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Editorial Policy. The Council has approved the following policy of management:

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1. The suitability of papers will not be judged by arbitrary standards of length but on their content of significant new research results in physiology, presented with the greatest brevity which is compatible with scientific accuracy and clarity.

2. Preference will be given to papers from American Laboratories in the field of vertebrate physiology and to those which contribute to problems related to this field.

3. Subdivision of material coming out of a general research into two or more papers will be discouraged.

4. Papers restricted to the description of new apparatus or methods or which appear to be of the nature of progress reports, the publication of which might properly be withheld until the research has progressed to the completion of at least a significant phase of the problem, will not be accepted.

5. Papers giving confirmatory or negative results will be considered only if presented in the briefest possible space.

The following practical aspects are important in the preparation of papers:

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- b. Tables and illustrative material should be prepared with the size of the Journal page ($4\frac{1}{2} \times 7\frac{1}{2}$ inches) in mind, specifically with the idea of conserving vertical space.

- c. It is advantageous, when feasible, to group illustrations. This should be done with as little waste space between the several units as is possible and also with the idea of conserving vertical space.

- d. Unless authors specify the desired reduction of their illustrations the printer will use his judgment in the matter.

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JULY 1, 1934

No. 1

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY
FORTY-SIXTH ANNUAL MEETING

New York, N. Y., March 28, 29, 30, 31, 1934

A new anatomic basis for the spread of the impulse in the mammalian ventricle.

D. I. ABRAMSON and J. C. CARDWELL (by invitation). Department of Physiology, Long Island College of Medicine. (Demonstration.)

The current view in relation to the conduction pathway in the heart postulates that the impulse, after spreading over the subendocardial Purkinje system, leaves this network to extend throughout the ventricular musculature by virtue of the conductivity of the myocardium itself. However, a study of the beef heart has proved conclusively that in the ventricular muscle are found Purkinje fibers which have as wide and diverse a ramification as the subendocardial network, and of which they are a direct continuation. The bundle of His, its two large subdivisions, as well as the Purkinje network, may be readily brought into view by means of injecting such materials as India ink and celloidin into the sheath surrounding the individual fibers.

The Purkinje system is arbitrarily divided into two subdivisions: 1, the subendocardial network which lies directly beneath the endocardium in the subendocardial areolar tissue, and 2, the myocardial network which is found in the muscle of the ventricles. Up to the present, very little physiologic significance has been given to this myocardial Purkinje system which forms plexus-like arrangements throughout the outer walls and septum of both ventricles, extending in many sites as far as the epicardial surface of the heart. In the outer wall of the left ventricle, these penetrating fasciculi are disposed in layers which run grossly perpendicular to the subendocardial network, whereas in the outer wall of the right, the layers are nearly parallel to the subendocardial network and to one another. Myocardial Purkinje fibers are found also in the interventricular septum, connecting the subendocardial system of the right ventricle with that of the left.

A grossly similar myocardial Purkinje network has been noted also in both the sheep and pig heart.

Anuria in the frog under the influence of carbon dioxide. EDWARD F. ADOLPH. Department of Physiology, School of Medicine and Dentistry, The University of Rochester. (Read by title.)

When a frog is placed in an atmosphere of 30 per cent CO₂ and 70 per cent oxygen usually no urine is formed. This is found by ligating the cloaca for a period of time while the frog is partly immersed in water; release of

the ligature at the end of a period of CO_2 liberates no urine. After the exposure (at 20° to $23^\circ\text{C}.$) diuresis develops, making up for the anuria within 2 or 3 hours. An atmosphere of 10 per cent CO_2 does not reduce the rate of urine formation.

An operated frog with tissues exposed generally requires a tension of CO_2 slightly higher than 30 per cent to produce comparable effects. When a pithed frog with one ureter cannulated is treated to CO_2 in a gas chamber, the flow of urine may slow within 2 minutes and ceases within 4 minutes, resuming within 10 minutes after room air is administered. In the exposed kidney of the pithed frog, which can be observed simultaneously with the ureteral output, it is seen that the blood may stop flowing through all the glomeruli in 1 to 4 minutes after the CO_2 atmosphere is furnished, returning in 2 to 8 minutes after room air is given. The flow of blood in the renal portal vein is not noticeably affected. With a critical tension of CO_2 in a given frog, some only of the glomeruli become reversibly inactive, allowing blood to flow through others.

Denervation of the kidneys does not prevent the anuria in the intact frog, nor does ligation of the renal portal veins. Subcutaneous administration of 1 per cent urea solutions or other fluids that tend to induce diuresis does not interfere with the anuria. The response to CO_2 is observed to be a constriction of the renal arterioles and afferent vessels, which is also the mode of response to lack of oxygen in the frog, as elsewhere reported. The stimulus to vasoconstriction evidently impinges within the kidney. In all the frogs observed under the influence of these two agents the rate of urine production is primarily correlated with the simultaneous flow of blood through glomeruli.

Levulose tolerance curves in renal diabetics. MARIE ANDERSCH (by invitation), B. B. CLARK (by invitation), and R. B. GIBSON. Pathological Chemistry Laboratory, The State University of Iowa, Iowa City, Iowa. (Read by title.)

In order to establish a possible basis for the differential diagnosis, glucose and levulose tolerance curves, using 50 grams loads of sugar, were run on normals, diabetics, and renal diabetics. It was found that on normal subjects, the levulose curves were relatively flat, as has been previously reported in the literature. Levulose administered to diabetics caused a hyperglycemia and glycosuria usually of similar magnitude or slightly less than the corresponding amount of glucose. In eight renal diabetics, the glucose curves indicated a slightly diminished tolerance. The blood sugar, however, usually returned to the base or fell below in two hours. Although the levulose curves were slightly higher in the renal diabetic than in the normal, they were appreciably lower than the glucose curve of the same object.

Serum inhibitory to the thyreotropic hormone. E. M. ANDERSON (by invitation) and J. B. COLLIP. McGill University, Montreal, Canada.

Animals injected with the thyreotropic hormone of the anterior pituitary show a temporary rise in metabolic rate which is followed by a depression of metabolism which may go as low as that of the hypophysectomized animal. This low metabolic rate can be raised, however, by the giving of thyroxine. The serum from these animals contains a substance which is capable of inhibiting the action of the thyreotropic hormone in normal and hypophysectomized animals.

The relation of the autonomic nervous system to the motility and secretion of the stomach in Elasmobranchs. B. P. BABKIN and M. H. FRIEDMAN. Department of Physiology, McGill University, Montreal.

This work was performed conjointly with Drs. M. E. MacKay-Sawyer and A. F. Chaisson at the Biological Station, St. Andrews, N. B.

Various branches of the visceral division of the vagus, when stimulated by an induction current of moderate strength, activate the local motility of different regions of the stomach. A stronger current usually produces a contraction of the pylorus, which gradually spreads to other parts of the stomach. Stimulation of the splanchnic nerves produces a much stronger contraction, which also begins at the pylorus and gradually moves towards the cardiac end. Contraction usually starts 12 to 20 seconds after the cessation of stimulation.

A fasting skate secretes continuously very small amounts of highly acid gastric juice. Section of the vagi or injection of large doses of atropin does not stop this secretion. Adrenalin inhibits this spontaneous secretion for a period of several hours. Stimulation of the sympathetic nervous supply to the stomach does not affect the secretion.

Elimination of the influence of the sympathetic nervous system by destruction of the spinal cord from the cervical region down to the tail results in a "paralytic secretion" of gastric juice. The amount of this secretion varies in different animals from a few cubic centimeters up to 30 cc. in 24 hours. The pH varies between 2.0 and 5.0 and the digestive power (Mett's method) is moderate. From the onset of the paralytic secretion to the end of the experiment the freezing point depression of the juice becomes gradually lower. This fall coincides with a lowering of the blood Δ . Since adrenalin stops the "paralytic secretion," it is highly probable that it is of purely vascular origin.

Relation of potassium to the contractions of mammalian skeletal muscle and its similarity to the effect of sympathetic stimulation. ANNA M. BAETJER.

Department of Physiology, School of Hygiene, Johns Hopkins University.

Stimulation of the sympathetic nerves temporarily diminishes the fatigue of contracting skeletal muscle (Orbeli). It has been assumed that this might be due to liberation of the adrenalin-like substance (sympathin) in the smooth muscle of the blood vessels. Because calcium has been shown to retard fatigue in frog muscle and because it has been claimed that calcium has an effect similar to sympathetic stimulation on some tissues it seemed desirable to study the effect of this ion.

Rhythmical contractions of the tibialis anticus in cats were produced by stimulation of the motor roots. Introduction into the arterial blood flowing to the muscle of 2 cc. of Ringer's solution was without effect on the contracting muscle if the concentration of CaCl_2 was 0.024–0.05 per cent but if the concentration was 0.05–0.65 per cent there was a temporary decrease in the contractions, due possibly to a vasoconstrictor effect.

On the contrary, introduction of a solution containing 0.2–0.8 per cent KCl always produced a temporary increase in the height of the contractions, proportional to the concentration of potassium. The effect was similar to that obtained with stimulation of the sympathetic but the progressive rise and subsequent return to the original height were much more rapid. Like the sympathetic effect it was not due to an alteration in the circulation and was unaffected by administration of ergotamine. The effect of potassium was undiminished when the muscle was stimulated

directly after curarization indicating that the effect is on the muscle fibers. Orbeli claimed that the effect of sympathetic stimulation is on the myoneural junction. It seems to disappear after curarization.

An attempt was made to determine if sympathetic stimulation caused the liberation of potassium. Analysis of the venous blood from the leg before and during stimulation of the lower abdominal sympathetic chain yielded inconclusive results, an increase being found in nine experiments (possibly due to vasoconstriction) and an insignificant increase in five.

Stimulation of the somatic nerves to the point where the muscle ceased to respond led to a marked increase in plasma potassium.

The effects of calcium chloride, acid and alkali, upon conduction in nerves locally cooled. DOMINIC BAIMA (by invitation) and HAROLD N. ETS. Loyola University, School of Medicine, Chicago.

Conduction in locally cooled sciatic nerves of frogs was studied by observing the temperature and type of block which developed. The frogs were kept in a refrigerator at 5°-8°C. for at least forty-eight hours before using. This treatment permitted conduction in freshly excised nerves to 4°-9°C. below zero; blocks occurred only when ice formed. The nerve only, directly after removal, was placed in the solutions investigated; the muscle was protected from the solutions and from drying.

Previously Ets and Boyd (This Journal 1933, 105, 31), had found that the blocking temperature of nerve treated with Ringer's solution containing an excess of potassium gradually rose until conduction failed at room temperature. The concentration of potassium and time of treatment were factors in the rate at which the effects developed. In contrast, nerves treated with isotonic solution of calcium chloride blocked only by freezing, after considerable supercooling. No change in type of block occurred, although with sufficiently long treatment the nerve became inexcitable at room temperature. Nerves treated with hydrochloric acid Ringer's solution, pH 3, or with sodium hydroxide Ringer's solution, pH 10, even for three hours, blocked by freezing. When Ringer's solution, pH 1.8, was used, blocking temperature of the nerves gradually rose with increased time of immersion until non-irritability developed in about 65 minutes. With the use of Ringer's solution, pH 12.5, the nerve still showed a tendency to block only by freezing; non-excitability resulted after 60 minutes of immersion. The preparations made non-excitably by calcium showed recovery after immersion in normal Ringer's solution. Neither the acid nor alkaline non-excitably nerves showed this recovery.

Localization of body vapor pressure reflexes to environmental temperature in the infundibular portion of the hypothalamus. HENRY G. BARBOUR. Department of Pharmacology and Toxicology, Yale University.

Reflex responses to environmental temperature by which the vapor pressure of the body varies in the direction favorable to heat regulation (decrease with cold, increase with warmth) have been described in detail by Gilman and myself. The serum osmotic pressure response to cold has now been studied in cats with a variety of transverse sections of the brain stem. Whenever the cat becomes poikilothermous, the osmotic pressure response to cold is abolished. The critical point for the osmotic pressure response is the infundibular region of the hypothalamus, long known as most important for heat regulation as well as for sympathetic control. Neither the supra-optic nuclei, nor other cell groups dorsal or anterior to

the tuberal region are essential to the reflex, which is abolished by complete transverse sections posterior to the mammillary bodies.

*Decorticate and decerebrate rigidities in the cat.*¹ PHILIP BARD, CHANDLER M. BROOKS (by invitation) and CLINTON N. WOOLSEY (by invitation). Departments of Physiology, Harvard Medical School and the Johns Hopkins University.

Complete decortication or bilateral removal of all cortex rostral to the ansate sulci (frontal poles) produces a permanent bilateral extensor rigidity. Permanent rigidities *confined to the contralateral limbs* result from the following procedures: 1, ablation of one frontal pole; 2, unilateral decortication; 3, unilateral removal of all tissue above the hypothalamus; 4, unilateral decerebration (removal down to intercollicular level). When in the same animal procedure 1 has been carried out on one side and either procedure 2 or 3 on the other side, the rigidities of the limbs of the two sides are always equal. When procedures 1 and 4 are thus combined the rigidity is slightly but definitely greater in the limbs opposite the unilateral decerebration. These results indicate that *the removal of a cortical influence is the most important factor in the development of decerebrate rigidity*. A mesencephalic influence is of definite but minor importance. The decorticate rigidities and the unilateral rigidities of surviving hemidecerebrate cats are revealed in full strength only when the animals are suspended and quiet; they do not interfere with locomotion and are scarcely detectable during standing.

Unilateral or bilateral removal of all neocortex situated caudal to the ansate sulcus does not produce rigidity. The small cortical remnants thus left successfully inhibit the development of decorticate rigidity. Rigidity has invariably failed to appear after cleanly circumscribed ablations of the medial third of the anterior sigmoid gyrus together with the gyrus preceus (removal of areas A, G, H and I of Langworthy). If this fraction of the cortex contains the premotor area (area frontalis) it can be said that removal of this area in the cat, as in the dog (Woolsey, Brain, Dec. 1933), is not responsible for the production of decorticate rigidity or any other form of hypertonia of the musculature of the limbs.

The effects of the endocrine glands on carbohydrate metabolism. A working hypothesis. B. O. BARNES. University of Chicago.

Previous work has shown that hypophysectomy or partial adrenalectomy prior to removal of the pancreas decreases the severity of experimental diabetes. Reports have been made that thyroid feeding mobilizes body glycogen and that hyperthyroidism aggravates diabetes while thyroidectomy improves that disease. It has been found that thyroid feeding to dogs with both the pituitary and pancreas removed did not produce any change in the glycosuria. The heart rate was increased but the polyuria and diarrhea usually found during thyroid feeding was not observed. By the injection of pituitary extracts into a hypophysectomized-pancreatectomized dog, a severe glycosuria can be produced. One possible explanation for all the above facts is the following: that the thyroid may exert an influence on carbohydrate metabolism through the pituitary which in turn may influence the adrenals. This is not a theory, but a working hypothesis which is being subjected to experimental test.

The physiological activity of the thyroid gland. B. O. BARNES, J. G. BUENO (by invitation) and MILDRED JONES (by invitation). University of Chicago. (Read by title.)

¹ This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

Various authors have reported that crystalline thyroxine is quantitatively less active than desiccated thyroid or thyroglobulin when doses containing equivalent amounts of iodine are administered, yet they are agreed that all of the iodine in thyroglobulin is not present as thyroxine. No definite evidence has appeared to indicate whether the discrepancy is due to other active iodine compounds or whether thyroxine in combination with other amino acids is more active than the crystalline form. Thyroglobulin has been split into acid-soluble and acid-insoluble fractions by treatment with pepsin for 12 to 24 hours. About one half of the iodine was present in each fraction. Analysis of these fractions for thyroxine by the method of Leland and Foster showed that the acid-insoluble fraction contained about 4 times as much thyroxine as the acid-soluble. Both fractions have been compared for physiological activity on 1, basal metabolism, weight loss, urine output and thirst in dogs; 2, weight loss in rats, and 3, the feather germ reaction for thyroid hormone.

The results by all these tests indicated that within the errors of the methods, the two fractions were about equal in activity. Furthermore, the rise in metabolism was approximately the same as was obtained when a dose of desiccated thyroid containing the same quantity of iodine was administered. If the acid-soluble material was further digested with commercial pancreatin, most of its physiological activity was lost although the thyroxine was still present.

Estimation of cardiac output from blood pressure measurements. H. C. BAZETT, F. S. COTTON (by invitation), L. B. LAPLACE (by invitation), and J. C. SCOTT (by invitation). Department of Physiology, University of Pennsylvania.

Blood pressures were measured by the method already described, and estimates of pulse wave velocities from the heart to subclavian, from the subclavian to the branchial and femoral, and from the femoral to dorsalis pedis were made. Cardiac output was determined by acetylene on the same day. Basal conditions were maintained. Through the courtesy of Doctors Starr and Gamble and their associates, comparisons were also made with the modified ethyl iodide method, though in this case the two sets of observations were conducted under somewhat different conditions. Repeated observations were made on 12 subjects of both sexes varying in age from 21 to 76, and in surface area from 1.36 to 1.89 with variations in systolic pressures from 94 to 205, and in pulse rates from 41 to 90.

In spite of the very varying types of subjects selected, the treatment of the blood pressure data adopted gave reasonable values for cardiac output; the average discrepancy between these estimates and those by acetylene was less than 15 per cent. Calculations by Broemser and Ranke's equations from data obtained by our methods agreed very poorly with respiratory estimates.

The calculations are based on the estimation of the outflow from the arterial tree during diastole from the fall of pressure succeeding the diastolic wave, from the distensibility of the system (as judged by the pulse wave velocities) and from the size of the vessels estimated from Suter's pathological data. The outflow in systole is estimated on the assumption that it is related to that in diastole, according to the relative pressures existing in, and the durations of, the two phases. The constants used have been adjusted empirically to give the best possible agreement.

We would like to express our thanks to Doctor Grollman for advice in regard to his method.

Estimation of lateral arterial pressure by the Riva Rocci method. H. C. BAZETT and L. B. LAPLACE (by invitation). Department of Physiology, University of Pennsylvania. (Demonstration.)

Records are demonstrated to illustrate the accuracy obtainable in estimating lateral arterial pressure by the optical Riva Rocci method previously described. Some records were obtained in a circulation schema, in which a section of the system was enclosed in a glass tube and was exposed to varying air pressures; others were obtained by the enclosure of a section of a dog's femoral artery in a similar system. Pressures were recorded directly with optical manometers (Wiggers type) with T-shaped cannulae inserted in the vessel above and below the compressed segment.

Records demonstrate that the lateral diastolic pressure is indicated by a change in the oscillometric curve, and that the initial negative wave described by Erlanger and by Bramwell is also developed at this pressure provided that the up stroke of the pulse wave is steep. The conditions of the development of the negative wave support Bramwell's hypothesis of a "breaker" effect. The error of estimation of diastolic pressure under favorable conditions is less than 3 mm. The pressure of the dicrotic wave may also be determined from the oscillogram with an error of some 5 mm.

At compressing pressures which exceed the diastolic level the systemic pressure is itself altered, and this interferes with the estimation of the systolic level, so that exact measurement of the pressure existing before compression has been impossible. The first occurrence of a peripheral pulse (which agrees with the criterion of the first sound) occurs at compression pressures above the unobstructed systolic level. The criterion we have suggested previously as probably significant for lateral pressure gives values which are too low.

Our thanks are due to Miss M. E. Maxfield and to Mr. N. Wander for their assistance in these experiments.

Evidence of sympathetic influence on anterior lymph hearts in Rana pipiens.

ERNEST B. BENSON (by invitation). Department of Physiology, Boston University School of Medicine. (Read by title.)

In the absence of all knowledge concerning a sympathetic supply to the walls of lymph hearts, an attempt was made to determine the effect of removal of the sympathetic chain in the region of these organs, the musculature of which consists of a meshwork of striated fibers rhythmically actuated by spinal nerves.

A portion of the sympathetic chain in the vicinity of the 3rd spinal nerve, including the rami communicantes, was removed on one side in the etherized animal by dorso-lateral approach from a point just posterior to the scapula. The time interval between operation and observation varied from 1 to 30 days. At the end of the period the hearts on each side were exposed under etherization by 1, raising the scapula to a vertical position; 2, cutting transversely the subscapular muscle to eliminate tension on lymph heart and blood vessels, and 3, removing the fascia covering the lymph heart. Observations were made under a dissecting binocular, and kymographic records of the contractions of both anterior hearts were ob-

tained through the use of light vertical straws attached to counterbalanced writing levers of equal length.

Up to a certain limit the amplitude of contraction of the heart on the operated side diminished progressively with lapse of time after operation, the results being consistent in 14 out of the 15 experiments. In one instance the aortic arches were clamped; in another, the blood-heart removed. Blank operation on the control side without sympathectomy did not, in any instance, alter the effect.

Results suggest the existence of a sympathetic supply to the anterior lymph hearts, with the possibility of a trophic interpretation of its significance.

Changes in peripheral blood flow accompanying localized exposure of carotid sinus region to low O₂ and high CO₂. THEODORE BERNTHAL. Department of Physiology, University of Michigan, Ann Arbor.

Changes in peripheral blood volume flow brought about reflexly by exposure of the carotid sinus and the carotid body of the dog to changing tensions of blood oxygen and to changing tensions of blood CO₂ were studied.

Each common carotid and each external carotid artery was cannulated. The internal carotid and the occipital arteries as well as all other carotid efferent branches were ligated, taking care to preserve the carotid sinus nerve and the blood supply of the carotid body.

The carotid sinuses and carotid bodies were then perfused with heparinized normal arterial blood at a constant mean blood pressure, constant pulse pressure and constant pulse rate. At intervals known changes in either the O₂ tension or the CO₂ tension of the perfusing blood were produced. Under these conditions the volume flow of blood in the axillary arteries was observed (Gesell's and Bronk's thermoelectric method), the blood flowing through these arteries also being supplied at a constant mean and pulse pressure and a constant pulse rate.

Lowering the O₂ tension of the blood supplying the sinus region by 10 mm. Hg or more was invariably followed by a diminished flow of blood through the axillary artery, usually well maintained until return to the original O₂ tension, whereupon the flow either returned to preadministration level or was temporarily increased above preadministration level.

Raising the CO₂ tension of the blood perfusing the sinus region likewise resulted in a decreased axillary blood flow which was often only partially or temporarily maintained during the period of high CO₂ administration. Upon resumption of the original CO₂ tension the axillary blood flow either returned to its original level or was temporarily increased above it.

These changes in axillary blood flow were strikingly accentuated by vagotomy. Changes of O₂ or CO₂ tension so small that they produced indefinite effects with vagi intact often produced marked effects after vagotomy.

The cause of action currents: A direct effect of the metabolic products independent of permeability changes. R. BEUTNER and J. J. PRUSMACK (by invitation). School of Medicine, University of Louisville.

Since excitation is generally associated with an increased permeability, it is customary to explain the origin of the negative electric variation coin-

cident with excitation as a result of that increased permeability. Yet in spite of extensive discussion continued for half a century, nothing is known about the nature and site of the respective membranes.

Through a valuable suggestion from Dr. H. C. Lawson of this school, we were led to inquire whether the negative variation may result from a variation of phase boundary potentials directly produced by any of the metabolic products which are known to arise in muscular contraction, such as lactic acid or creatin, or possibly adenine, inosinic acid or phosphoric acid.

To test this idea we used the apparatus described by one of us (see R. Beutner, *Physical Chemistry of Living Tissues and Life Processes*, Baltimore, 1933, p. 202), where a massive layer of a fat, with or without addition of a water-immiscible solvent, like guaiacol, etc., is inserted as an electrical conductor between two watery solutions. Electric connections from these solutions to a measuring instrument are made by impolarizable electrodes (compare fig. 59, in the book mentioned). The composition of one of these two solutions can be changed. The effect of any such change upon the electromotive force of the entire system is then observed.

When using as a central conductor in this apparatus lecithin or lecithin + guaiacol in contact on one side with physiological saline, the addition of lactic acid to that saline resulted in a distinct negative variation (data to be presented). Since our central layer (or "membrane") is 1 cm. thick the possibility of a change of permeability is excluded. It appears, therefore, that metabolic products can produce a negative variation in contact with lipoids even if permeability has no influence.

The details of the time curve of the action current of a single nerve or muscle fibre ought to be explained, we believe, as resulting from the chemical changes involved in excitation and possibly in recovery.

New experiments pleading against the pore theory of Michaelis. R. BEUTNER, J. J. PRUSMACK (by invitation), and N. EPSTEIN (by invitation). School of Medicine, University of Louisville. (Read by title.)

The following observations plead against the pore theory of L. Michaelis according to which positive and negative ions acquire a difference of mobility in membrane pores and give rise to diffusion potentials.

The following cell arrangement was measured:

liquid collodion ether and alcohol added	solid dried collodion	KCl of varying concentration
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If the KCl concentration was changed the electromotive force varied in the same manner as on an ordinary dried collodion film, no matter which solution was first applied. This finding would be inconceivable according to the pore theory. The filling of the pores with KCl solution from opposite sides is manifestly obviated in this arrangement, hence no influence of the concentration upon the electromotive force of this system should appear according to the pore theory. This is never the case.

This discrepancy may perhaps be explained by a filling of the pores on their first contact with a given KCl solution which stays in the pores and gives rise to diffusion potentials.

If this explanation is correct, the electromotive force ought to depend on the concentration of the solution which was first applied, but experiments have shown that this is by no means the case.

Another argument against the pore theory is found through observations with mixed solutions. The potential of a binary mixture of two salts in solution is about near the middle value in the case of diffusion potentials, but in case of phaseboundary potentials it is not near the middle value, but almost equals the potential produced by the more penetrating of the two salts alone. This can be demonstrated by experiments and conforms to theoretical expectations.

The potential differences at collodium films follow the rules established for phaseboundary potentials in this respect. It is difficult to see, therefore, how they can be diffusion potentials as claimed by Michaelis.

A compromise between the two alternatives, viz., something between phaseboundary potential and diffusion potential is manifestly impossible.

Metabolic rates of cat-fish treated with thyreotropic hormone. P. T. BLACK (by invitation). McGill University, Montreal.

Cat-fish 4 to 5 inches long, kept in 5 to 12 litres of aerated water for 24 hours, showed markedly increased oxygen consumption and nitrogen and CO₂ excretion. Injected fish placed in running water showed no increase in oxygen consumption. Normal fish placed in water in which injected fish had been kept for a period of 24 hours also showed increased metabolic rates. It appears that injected fish secrete or excrete a substance into water which stimulates metabolism.

On addition of local excitatory processes. H. A. BLAIR (by invitation). School of Medicine and Dentistry, University of Rochester.

Using three electrodes *a*, *b* and *c*, on the sciatic nerve of the frog the direct current rheobase may be determined with *a* and *c* as anodes, with *a* alone as anode, or with *c* alone as anode, *b* being cathode always. Three rheobase voltages *Rabc*, *Rab* and *Rbc* are thus obtained. The quantities *Rabc/Rab* and *Rabc/Rbc* respectively are the ratios of the currents in each direction when both are flowing to when but one is flowing. These ratios are less than unity. Therefore in a particular direction less current is adequate when there is also a current in the other direction. By putting series of resistances in either branch each of the ratios can be given values ranging from nearly unity to nearly zero. If the components of each pair are plotted against each other the resulting points fall along the lines joining the coördinates (1, 1) to the coördinates (2/3, 2/3) approximately. According to a theory of excitation in which the local excitatory process is proportional to the current when the current has been flowing for rheobasic time will *Rabc/Rab* equal the fraction *Eab* of the adequate excitatory process existing in the branch *ab* and will *Rabc/Rbc* equal the fraction *Ebc* of the adequate excitatory process existing in the branch *bc*? The data therefore conform to a relation,

$$Eab + qEbc = 1 \text{ or } Ebc + qEab = 1$$

where *q* is a constant. The interpretation is that the seat of the impulse is at the greater of the local excitatory process but that a constant fraction of the lesser is added to the greater to make it adequate.

The importance of decreased cardiac work in the relief of angina pectoris by total ablation of the thyroid. HERRMAN L. BLUMGART and DAVID D. BERLIN (by invitation). Department of Medicine, Harvard Medical School, and the Research Laboratories of the Beth Israel Hospital, Boston.

Over six hundred measurements of the velocity of blood flow through the lungs of man have shown that the speed of blood flow is an important gauge of the circulation and varies according to the metabolic rate. When the thyroid is overactive as in thyrotoxicosis, the basal metabolic rate is elevated and the speed of blood flow is correspondingly increased. In myxedema, the thyroid is relatively quiescent, the basal metabolic rate is reduced, and the speed of blood flow is slowed. This circulatory slowing in myxedema is accompanied by decreased cardiac output, a lower ventricular rate, and signifies a decreased amount of work.

When the work of the heart is augmented as in exercise, there must be a rise in the coronary circulation. If, because of arteriosclerotic narrowing or other causes, the coronary circulation cannot increase in accord with the increased needs of the heart, relative anoxemia results and angina pectoris occurs. In over twenty patients with angina pectoris the entire thyroid gland has been removed and a state of hypothyroidism has been regularly produced. Since the heart after complete thyroidectomy performs less work and starts at a lower level of oxygen consumption, it can withstand a greater increment of work before reaching the upper limit of oxygen supply set by the relatively fixed coronary vessels. The striking relief afforded our patients by complete thyroidectomy is in accord with these considerations for the extent of relief has been related to the degree of decrease in the metabolic rate. Whether the decrease in cardiac work at a low metabolic rate is due to decreased velocity of blood flow or cardiac output, a lowered ventricular rate, a lowered blood pressure or to decreased sensitivity to epinephrin has been investigated. Of these factors, the circulatory rate has shown the most striking change.

Some patients have experienced temporary relief from angina pectoris immediately after operation while the metabolic rate was unchanged. This early transient relief is due to interruption of nerve impulses from the heart and plays little or no rôle in the permanent improvement shown by our patients.

The action of carbon monoxide on the respiration of normal and blocked embryonic cells (Orthoptera). J. H. BODINE and E. J. BOELL (by invitation). Zoological Laboratory, State University of Iowa. (Read by title.)

Two types of response to the action of carbon monoxide are noted in the respiration of the embryo of the grasshopper, *Melanoplus differentialis*.

The respiratory rate of embryonic cells in a state of normally occurring physiological block or diapause is stimulated in a carbon monoxide-oxygen medium in which the oxygen tension is sufficient to sustain normal respiration. Increasing the concentration of CO and correspondingly decreasing the O₂ tension depresses the respiratory rate, but the degree of depression is, within the limits of error, of the same magnitude as that obtained in nitrogen-oxygen mixtures of similar concentrations.

The oxygen consumption of physiologically active embryonic cells, on the other hand, is considerably depressed by CO/O₂ mixtures, the degree

of depression depending upon the concentration of CO. At sub-minimal oxygen tensions the depression is always much greater than in nitrogen-oxygen mixtures of similar oxygen concentrations.

Attention is directed to the fact that recognition of the physiological state of the experimental material may serve to clarify the seemingly discrepant results reported in the literature relative to stimulation and depression of O₂ consumption by carbon monoxide.

Liver glycogen changes in fed and fasted animals before and after abdominal denervation. R. C. BODO, F. W. CoTUI (by invitation), A. E. BENAGLIA (by invitation) and M. M. FRIEDMAN (by invitation). The Departments of Pharmacology and Experimental Surgery, University and Bellevue Hospital Medical College, New York City.

The following experiments were carried out on dogs fed or fasted for seven days. After a subcutaneous injection of morphine sulphate (3 mgm./kgm.) laparotomy was performed under local anesthesia, liver samples taken for glycogen determination and the abdominal opening sutured. The animals showed no signs of discomfort or pain during the operation, being usually asleep. During the subsequent five hours, at half hour intervals, dextrose solution was injected subcutaneously into the fed animals and saline solution into the fasted ones. The injection of morphine was repeated once or twice during the five hour period. Blood samples for sugar and lactic acid determinations were taken throughout the whole experiment half hourly. The final liver samples were taken either in local anesthesia or immediately after an intravenous injection of chloroform.

The results were as follows: In the fed animals in spite of the dextrose injections there was a decrease in liver glycogen, a decrease in muscle glycogen, an increase in blood lactic acid and a hyperglycemia starting before the injection of dextrose. In the fasted animals though given only the saline injections there was an increase in liver glycogen, a decrease in muscle glycogen, an increase in blood lactic acid and hyperglycemia throughout the experiment.

When previous to the experiment, under sterile conditions and in successive stages, the entire abdominal sympathetic chains were removed bilaterally, all the splanchnic nerves cut bilaterally and all the abdominal branches of both vagi severed, the results obtained in the actual experiment were entirely different and were as follows:

Practically no change in blood sugar or blood lactic acid following the injection of morphine and the laparotomy, an increase in liver glycogen in the fed animals when dextrose was given and no increase in liver glycogen in the fasted animals when saline was given.

When adrenalin was injected into denervated fed animals the same changes were brought about as were observed in the animals with intact sympathetic and vagus nerves.

Natural dry digestive juices, their properties and use in laboratory and clinic. W. N. BOLDYREFF (by invitation). The Battle Creek Sanitarium, Battle Creek, Michigan.

Instead of drawing digestive ferments from dead organs and instead of using liquid juices, the author offers to prepare them in a dry state. Various

juices (saliva, gastric juice, pancreatic juice, bile, intestinal juice, etc.) are gathered aseptically if possible, directly from the fistulae into sterile receptacles, cooled by CO₂-ice. If they are not pure they are first filtered through a porcelain filter (Chamberland's or other) with a low temperature (about 10°C. or lower). Filtration must be made as quickly as possible, and immediately after that the juice must be frozen. In hard frozen state it is transferred into the desiccator from which all air is pumped out with a powerful vacuum-pump. If necessary, two vacuums may be used, connected in a series to obtain a pressure near 0. Then, during several hours, the juice is drying, without melting. In this manner all ferments are saved without loss and apparently the properties of the juices undergo no changes. The author has tried out all juices mentioned. Dry juices are quickly dissolved in distilled water (in about 1 minute) (the gastric juice in 0.5 per cent HCl). Comparing the juices before and after drying, the author found the power of ferment action unchanged (the following were tested: invertin, pepsin, trypsin, amylopsin, pancreatic lipase). The dried juices must be kept hermetically sealed in glass vessels in a dark cool place. They keep practically for an indefinite time. Such juices do not require bulky breakable glass containers and can be easily sent by mail (small amounts may even be enclosed in ordinary letters) and may be used any time.

The influence of infection with slight elevation of temperature on the nitrogen balance. WALTER M. BOOTHBY, MILDRED ADAMS (by invitation) and JESSE L. BOLLMAN. Institute of Experimental Medicine and the Section of Clinical Metabolism, Mayo Foundation and Mayo Clinic, Rochester, Minnesota.

In a balanced metabolism experiment the following determinations were made: 1. The food intake was made up and analyzed weekly for nitrogen, sulfur, phosphorus, calcium, magnesium, potassium and sodium. Its heat value was determined in a bomb calorimeter and it was also burned in a combustion tube in such a manner that its respiratory quotient was determined. 2. The urine was analyzed for all the nitrogenous constituents and also for the inorganic. 3. The feces (including hair and cage washings) were analyzed for nitrogen, sulfur, phosphorus, calcium and magnesium. 4. The heat production and respiratory quotient under basal conditions, working on a treadmill and for the total twenty-four hours, were determined for a period of nine weeks on a female dog weighing about 15 kgm.

During the course of the experiment the dog spontaneously became ill with elevation of temperature—found at autopsy to be due to a severe endometritis and salpingitis. When the illness began the dog was gaining weight and was in positive nitrogen balance. The daily temperature had been around 99.8°F (rectal). Vaginal discharge developed and temperature rose to 100.3°F and remained there for three weeks before increasing to over 103°.

The most interesting and important observation was that a chronic infection with average elevation of temperature of 0.5°F. changed an average daily positive nitrogen balance of 0.4 gram with gain in weight to an average negative nitrogen balance of 1.3 grams with loss in weight while the dog still continued to eat all her food. After three weeks, when the temperature rose to over 103°, she stopped eating and, of course, the

negative nitrogen balance and loss in weight became much larger as always occurs in fasting. The basal heat production and respiratory quotient were unchanged until the temperature increased to 103°. At this time, the basal heat production increased, roughly corresponding to Van't Hoff's law; the total daily heat production decreased due to less movement in the respiration chamber.

Reversible and non-reversible freezing block in frog nerve. T. E. BOYD and I. F. HUMMON, Jr. (by invitation). Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago. (Demonstration.)

Local cooling is produced by allowing either a cold salt solution or expanding CO₂ gas to pass through a metal tube across which the nerve lies. The nerve may be supercooled by protecting it against contact with water of condensation. A Ringer-soaked nerve conducts until ice forms, but after blocking at low temperatures (-5.0 to -10°C.) it usually fails to recover on being warmed. Conduction may return if the nerve is soaked for a time in Ringer's solution.

Supercooling may be minimized by allowing water to condense on adjacent surfaces in contact with the nerve. When the temperature is just below 0°C., freezing of the water film is started either by applying a small piece of ice or by arranging such a temperature gradient that crystallization begins spontaneously at a distance. When freezing spreads to the nerve it becomes blocked. The cooling need not be lower than -1.0 or -1.5°C., and recovery on warming is immediate.

The variation of electrical resistance of muscle during contraction. E. BOZLER. Johnson Foundation, University of Pennsylvania.

The resistance to alternating current (1000 to 10,000 cycles) of frog's sartorius muscle and the tension developed were recorded simultaneously. The current passed through the muscle vertically and its variation due to resistance changes was recorded by a string galvanometer after amplification and rectification. The sources of error due to movement of the muscle during contraction were studied. It has been found that they can be avoided by using small electrodes and holding firm the region of the muscle surrounding them by gentle pressure. The resistance increases during the contraction 5 to 10 per cent in a maximal twitch. The resistance change follows quite closely the change in tension. It has, however, a slow phase which persists for several seconds and which becomes much larger after some contractions, even though the tension record is still unchanged. In a tetanic contraction the resistance change is summated as is the tension.

Reflex closure of the glottis by stimulation of afferent (visceral) nerves.

NATHAN BREWER (by invitation), D. S. BRYANT (by invitation), and A. B. LUCKHARDT. Physiological Laboratory of the University of Chicago.

In 40 dogs under either barbital, ether, or paraldehyde anesthesia, observations were made on the vocal cords from the oral side and by opening the trachea just below the thyroid cartilage and viewing the cords from below. Records of the variations in the laryngeal size were made by means of a balloon placed between the vocal cords and connected with a

water manometer. Action currents were obtained from one of the recurrent nerves (in a few experiments), during light visceral traction and during stimulation of the left splanchnic nerve.

It was found that traction on the abdominal viscera produced a cessation of respiration and a marked contraction of the vocal cords (adductors). Stimulation of the central end of the left splanchnic produced the same result, but of a stronger degree.

When respiratory movements did break through, the tone of the vocal cords, as judged by the relatively feeble abductions on inspiration as well as the degree of adduction on expiration, was increased. This was substantiated by action potential records.

A partial or complete adductor spasm may often occur during abdominal operations leading to interference with ready gaseous exchange in the lungs (asphyxiation).

Further studies on reproduction and cortico-adrenal function. S. W. BRITTON and R. KLINE (by invitation). Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

In an experimental group of 64 normal female and 16 adrenalectomized male rats, 35 animals became pregnant in a ten-week observation period and gave birth to normal litters. The males which survived the operation and retained potency were in every case found to possess accessory adrenal tissue. In a control group of unoperated animals there were 44 pregnancies during the same period. The fertility in the experimental group was approximately 20 per cent lower than in the controls.

Adrenalectomized females mated with normal males became pregnant in less than 10 per cent of 64 cases observed. Accessory adrenals were found in all the fertile cases at autopsy.

Pregnant rats at different stages of gestation which were subjected to adrenalectomy invariably aborted or resorbed their young, and usually died within two weeks of operation. Pregnancy and parturition were observed to follow a normal course, however, in the case of adrenalectomized animals which were given daily injections of cortico-adrenal extract. The importance of cortico-adrenal relationships to pregnancy, parturition and lactation is further apparent in experiments in progress.

The discharge of sympathetic impulses from the stellate ganglion and its relation to cardiac reflexes. D. W. BRONK, L. K. FERGUSON (by invitation), and D. Y. SOLANDT (by invitation). The Johnson Foundation, University of Pennsylvania.

Efferent impulses in sympathetic nerves running to the heart from the stellate ganglia have been recorded by means of an amplifier and oscillograph. Generally there is a continuous discharge of impulses which are grouped into irregular and random waves of considerable magnitude. It has been found that these waves are fairly synchronous in the nerves from both the right and left ganglia thereby indicating that the grouping of impulses is of central origin.

Under certain conditions the impulses group into waves which are synchronous with the pulse. This relationship immediately disappears if the carotid sinus and aortic nerves are cut. There may also be a marked respiratory rhythm with inhibition of impulses during inspiration. Such a rhythm is abolished by section of the vagi. The sympathetic discharge

is therefore under a very definite reflex control. Distention of one carotid sinus, for instance, inhibits the discharge from either the right or left ganglion. The degree and duration of this inhibition is a function of the level of pressure within the sinus. Simultaneous distention of both sinuses results in sympathetic inhibition for a period equal to the sum of the periods resulting from the distention of each sinus alone. Such inhibition is considerably lengthened by previous section of the aortic nerves.

The pressure-tension-temperature relation in cardiac muscle. DUGALD E. S. BROWN. Physiology Department, New York University and Bellevue Hospital Medical College.

When the auricular muscle of the turtle is subjected to a constant hydrostatic pressure at, for example, 210 atmospheres, the tension developed in the isometric twitch is found to vary as a linear function of the temperature over the temperature range from 2°C. to 20°C. Over this temperature range the tension-temperature coefficient; that is, the unit increase in tension per degree temperature, increases at pressures as high as 400 atmospheres. If the muscle is subjected to a series of pressures from 68 to 400 atmospheres at temperatures of 0°, 5°, 10°, 15°, and 20°C., the tension is found to increase as a function of pressure above 10°. At temperatures from 5° to 8°, the tension is practically unchanged; whereas, at temperatures below 5°C., the tension decreases as a function of pressure. The pressure-tension coefficient; that is, the unit change in tension per atmosphere of pressure, is zero at from 5° to 8°; at temperatures above 10° the value is positive and increases with the temperature up to 20°; while below 5° the value is negative and increases as the temperature decreases. By a suitable treatment, a simplified expression of the pressure-tension-temperature relation is obtained. In their more general aspect, the results are in agreement with the earlier studies on the turtle ventricle, carried out by Edwards and Cattell.

Respiratory metabolism of infrahuman primates. M. J. BRUHN (by invitation). Laboratory of Physiology, Yale University.

The respiratory metabolism of 17 infrahuman primates: monkey, baboon, gibbon, orang outan and chimpanzee was studied by means of a respiratory chamber ventilated by the Benedict Universal Respiration Apparatus. Each animal was 18 hours post absorptive when used in an experiment. To obtain minimum activity experiments were performed at night when animals would normally be asleep. The surface area of the monkey and the baboon was estimated by means of the Meeh formula $S = K \times W^{2/3}$ in which $K = 11.7$. The surface area of the anthropoid apes was actually measured by the original method of DuBois and DuBois.

It was found that when measured at an environmental temperature of 23°C. the adolescent rhesus and the immature mangabey monkeys gave a metabolic rate of approximately 650 calories per square meter of body surface in 24 hours. The baboons which were adolescent gave a higher rate of 748 calories, and the immature gibbon the low rate of 613 calories. The orang outan and the chimpanzee, all of which were adolescent, averaged 788 and 779 calories respectively per day.

Mangabey and rhesus monkeys from which the frontal cortex had been removed either unilaterally or bilaterally showed little or no change in the metabolic rate. One rhesus monkey which had increased rapidly in

weight following a hypothalamic lesion gave an average metabolic rate of 574 calories. Two rhesus monkeys from which the ovaries had been removed averaged 584 calories per day.

Before it is possible to interpret the results of the experiments on operated animals the effect of age, environmental temperature, seasonal changes, and sex upon the metabolic rate of the normal animal will have to be determined.

A mechanical device which produces a uniform dispersion of leucocytes in the diluting papilli and increases the accuracy of the leucocyte count. W. RAY BRYAN (by invitation). Medical School, Vanderbilt University. (Demonstration.)

Demonstration made with explanatory charts.

The use of hypertonic sucrose solution to reduce cerebrospinal fluid pressure without a secondary rise. L. T. BULLOCK (by invitation), R. KINNEY (by invitation) and M. I. GREGERSEN. Laboratories of Physiology in the Harvard Medical School.

Both clinical and experimental evidence proves that the use of hypertonic sodium chloride and glucose solutions for reduction of cerebrospinal fluid pressure is followed by an undesirable secondary rise in the pressure. We have attempted to overcome this undesirable effect by using sucrose. When injected intravenously, this substance is non-toxic, has a high osmotic pressure and is quantitatively excreted by the kidney together with a considerable volume of body water (Keith).

In experiments performed on dogs under sodium amytal anesthesia, cerebrospinal fluid pressures were taken in the cisterna magna for 10 to 13 hours. Continuous blood-pressure records were obtained from the femoral artery; urine volume, respiration, heart rate and rectal temperature were noted every 15 minutes. A 50 per cent sterile sucrose solution was injected intravenously at 5 cc. per minute in doses of 3 to 8 grams per kilo body weight.

Control experiments without sucrose injection all showed a slow, steady rise of cerebrospinal fluid pressure during the period of observation. There was also a moderate increase in the number of white cells in the spinal fluid. This increase was not produced by sodium amytal alone.

In experiments with sucrose the normal, control pressure was observed for 1 hour or more prior to the injection. The maximum reduction of cerebrospinal fluid pressure occurred within an hour after injection began. The extent of the reduction (50 to 150 mm. of Ringer's solution) was dependent upon the amount of sucrose given and upon the height of the initial pressure. Seemingly, the effect was greater with higher initial pressures. The fall of cerebrospinal fluid pressure was followed during 5 to 7 hours by a gradual rise, occasionally to normal but more frequently to a subnormal plateau. Although the pressure was often observed for 12 hours after injection, there was no indication of the beginning of a secondary rise except in 3 experiments. In these, the terminal rise could be ascribed to definite causes other than sucrose.

Injection was followed by a period of active diuresis lasting 3 hours, during which most of the sucrose was eliminated together with a volume of urine 4 times the volume of fluid injected. With doses of 6 grams per kilo,

the body fluid lost through the kidney was 4 to 5 per cent of the total body weight.

The absence of a secondary rise of cerebrospinal fluid pressure after sucrose may be explained by the excretion of the agents necessary to produce it, namely, the injected substance (sucrose) and free water.

Experiments with sodium chloride and glucose under the same conditions confirmed the experience of other investigators that both substances, more especially sodium chloride, produce a terminal rise of pressure which far exceeds the original level.

The effect of hexoses on the metabolism of alcohol in man. THORNE M. CARPENTER, MARION BURDETT (by invitation) and ROBERT C. LEE (by invitation). Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.

The respiratory exchange and the alcohol in the expired air of a human subject were measured in 15-minute periods for at least three hours after the ingestion of alcohol alone and after alcohol with an iso-molecular quantity of glucose, fructose, or galactose. Alcohol disappeared from the expired air most rapidly when it was ingested with either glucose or fructose, more slowly when alcohol alone was given, and most slowly when it was ingested with galactose. The depression of the R.Q. after the ingestion of alcohol with sugars was nearly the same for the first two hours as it was when alcohol alone was given and in the third hour the R.Q. tended to return to the same level as the R.Q. did when the sugars alone were given. When repeated doses of glucose or of fructose were given at hourly intervals and alcohol was taken at the beginning of the third hour, there was a marked depression for two hours in the R.Q. from the high level that was obtained when the sugars alone were given in the same manner, with a return to nearly the same high level in the latter part of the third hour after ingestion of alcohol that was found when the sugars were given alone in repeated doses. The simultaneous ingestion of either glucose or fructose accelerated the metabolism of alcohol and the metabolism of the sugars and of alcohol took place simultaneously, as was shown by the return of the R.Q. to the same levels after the alcohol had nearly disappeared from the expired air that were obtained with the R.Q. after the ingestion of the sugars without alcohol.

Changes in the efficiency of muscular contraction under pressure. MCKEEN CATTELL. The Department of Physiology, Cornell University Medical College, New York City.

The relationship between the tension and initial heat in the single twitch of muscles contracting under various degrees of hydrostatic pressure has been studied. Double sartorius preparations from the frog were mounted on a thermopile and tension lever system and completely immersed in paraffin oil, through which the pressure was applied. The observations were made at temperatures between 6° and 8°C. Pressures up to about 2000 pounds per square inch result in an increase in the tension of the twitch averaging about 30 per cent accompanied by a corresponding increase in the heat production. As the pressure is raised further the heat increases faster and reaches its maximum value at a higher pressure than the tension, following which both the heat and tension values fall and finally give values smaller than the controls taken at atmospheric pressure.

Since the heat values are always better maintained than the tension, it is possible in every preparation to select a pressure which causes a depression in the tension and at the same time augments the heat production, thus giving a reduction in the T/H ratio, which averaged about 25 per cent. As the pressure reaches a value of about 5000 pounds there is a sharp reduction in both tension and heat without further change in efficiency. These effects are completely reversible. The sequence of changes described above is obtainable in all preparations, but the pressures at which they occur are subject to wide variation depending upon the condition of the muscle, the temperature, and other factors. These results indicate a primary stimulation of the energy yielding processes, followed at higher pressures by depression. At intermediate grades of pressure the drop in efficiency suggests some interference with the mechanism concerned in the development of elastic potential such as would be imposed by an increase in viscosity.

The action of intense sonic vibration on pepsin and trypsin. LESLIE A. CHAMBERS (by invitation). Johnson Foundation, University of Pennsylvania.

The influence of 9000 cycle vibrations, produced by the Gaines magnetostriction oscillator, on various types of pepsin and trypsin extracts and concentrates was studied. Samples were exposed to vibration for varying time periods up to 30 minutes. In all cases there was a rapid decrease in proteolytic activity of the enzymes. Inactivation of pepsin is interpreted as thermal denaturation. The inactivated trypsin was found to differ from heat denatured material in that the inactivation was irreversible.

Some types of both pepsin and trypsin preparations showed an initial increase in activity reaching a peak value after about 2 minutes of vibration. The degree of activation varied from one type of extract or concentrate to another. Activity as measured by digestive power rose from 25 per cent to 250 per cent over unvibrated samples. No increased activity was found in the case of Northrup's crystalline pepsin preparation.

The activation of enzyme preparations by vibration is probably due to the dispersion of molecular aggregates resulting in increased specific surface. Such dispersive action of sound has been established. Enzyme preparations prepared by different means apparently show different degrees of molecular association.

Glycerol was found to protect pepsin against both activation and inactivation by vibration. The degree of protection is a function of the glycerol concentration, and, probably more directly, of the viscosity of the solvent mixture.

Inhibition of secretory activity of kidney tubules in tissue culture. R. CHAMBERS, M. BELKIN (by invitation) and L. V. BECK (by invitation). New York University.

Isolated proximal tubules of the chick mesonephros survive and function in tissue culture. The test for function is the passage of phenol red from the surrounding medium and its accumulation in the lumina of the blind tubules.

Preparations were exposed to various reagents in Tyrode with pH maintained between 7.4 and 7.6, and effects noted on the uptake of phenol red. The following results were secured: 1. HCN and H₂S, in M/5000 to M/1000 concentrations, completely and reversibly inhibited accumulation

of phenol red. 2. Ethyl urethane, in 1.5 per cent and lower concentrations, and phenyl urethane in $\frac{1}{2}$ saturated and lower concentrations, caused decided increase in rate of phenol red accumulation over that of the controls. With higher concentrations (6 per cent ethyl urethane, saturated phenyl urethane) inhibition occurred, which in some cases was reversible. 3. Iodoacetate, in concentrations between M/5000 and M/1000, markedly inhibited phenol red accumulation. This effect was reversible, provided the preparation had not been exposed too long (less than 30 minutes). The presence of lactate, in molar concentration 10 times that of the iodoacetate, decreased the inhibition by iodoacetate. This neutralizing effect was most marked when the preparations had been immersed in lactate-Tyrode for some time (about 30 min.) before immersion in iodoacetate-lactate-Tyrode.

These results suggest that the reactions from which energy is derived for phenol red secretion are chiefly those concerned in oxygen activation processes, and that lactic acid formation is probably involved.

A comparative study of hemolysis of red blood corpuscles of normal and thyroid-fed dogs. STEPHEN CHANG (by invitation). University of Chicago.

Samples of blood were drawn from the superficial leg vein of eight dogs once a day for six days, after which period the animals were fed one gram of desiccated thyroid per kilogram of body weight for six days. Beginning from the sixth day of thyroid-feeding, a sample of blood was drawn from each dog every day for another six-day period while the animals still received the thyroid. Another sample was drawn from each dog after ten days had been allowed for recovery.

For determining the percentage of hemolysis a series of six hypotonic sodium chloride solutions was used, varying by 2 millimols in concentration, from 58 to 68 millimols. The solutions were made at a pH of 7.4 by using secondary sodium phosphate and the work was performed with the solutions at a temperature of 30°C.

The number of grams of hemoglobin in each solution was determined with a Newcomber hemoglobinometer. The numbers of hemoglobin liberated by the red corpuscles in the salt solutions were compared with the total hemoglobin of the blood to determine the percentage of hemolysis.

The results of this experiment shows that the solutions used have a greater hemolysing effect (10 per cent to 20 per cent) on the red corpuscles during the period of thyroid feeding.

Increased effectiveness of insulin when given day and night to diabetic patients by injections of equal unitage at intervals of 2 to 4 hours. B. B. CLARK (by invitation), R. B. GIBSON, and R. G. SNYDER (by invitation). Pathological Chemistry Laboratory, The State University of Iowa, Iowa City, Iowa.

Insulin was administered to a group of 11 non-complicated diabetic patients in 1929 in equal unitage injections day and night irrespective of meals. It was found that the insulin requirement was diminished so that a half to two-thirds of the previous dosage sufficed to keep the patients' urine sugar free and the blood sugar at a reasonable figure. Night hypoglycemic reactions were avoided by omitting one injection, usually at midnight. Up to the present a total of 146 cases have been so treated, 74

of which were uncomplicated severe diabetics, and 72 with complications usually surgical. The procedure has been of great value in managing very severe uncomplicated diabetics, in treating diabetics with acute infections, and in handling post-operative cases.

Blood sugar values are somewhat high after breakfast, lower after the noon meal and supper, and quite low during the night hours. Thus a patient on a Woodyatt type diet and receiving 7 units of insulin every 3 hours including midnight had the following blood sugars: 9 a.m. 183 mgm., 2 p.m. 107 mgm., 7 p.m. 131 mgm., 12 p.m. 37 mgm., 7 a.m. 44 mgm., and 9 a.m. 167 mgm. (meals at 7 a.m., 12 noon, and 5 p.m.), without any hypoglycemic symptoms. Patients are more likely to have hypoglycemic reactions during the daytime or early evening in spite of low blood sugars obtaining at night and the reactions are usually not severe.

As the patient's tolerance improves, the insulin dosage must be reduced. However, when insulin given in two or three injections a day is resumed, uncomplicated cases require either the same or, more often, more insulin. Post-operative cases showing rapid improvement as the result of operation require either the same number of units or less when taken off of divided dosage.

The data suggest a rather constant and continuous secretion of insulin on the part of the islet cells of the pancreas.

*Oxidizing enzymes from brain.*¹ M. B. COHEN (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago. (Read by title.)

Brain cells, because of their high enzyme content and easy formation of homogeneous sols, offer excellent material for the study of enzyme activity. An extract, prepared by cytolyzing rabbit brain with distilled water and centrifuging, possesses the properties of a colloidal sol, is cell-free and contains little debris.

It has the following properties: 1. A small residual respiration—destroyed by heating, lost in weeks at 5°C., inhibited by neutral red—which falls off to zero in 4 to 5 hours at 37°. The cmm. O₂ consumed per milligram dry weight per hour average 0.5 for the first 4 hours (compare with 5 for brain slices). 2. Filtration through porcelain decreases respiration by about $\frac{1}{3}$ but does not materially affect its other properties. 3. The R.Q. of the residual respiration is 0.5. 4. pH variation from 7.2 to 8.0 has little effect. 5. The residue from the original centrifugation, when washed and added to extract or filtered extract, increases its respiration 20 per cent above the sum of the two respirations, the synergic effect being abolished by heating either extract or residue.

6. The extract readily dehydrogenates (respiration increased 20 times) succinate, paraphenylenediamine, methyl glyoxal, glycerophosphate slightly, but not glucose, fructose, lactate or pyruvate; oleate and citrate inhibit the respiration 30 to 50 per cent. These dehydrogenations proceed even after residual respiration stops. 7. Cyanide inhibits oxidation of succinate, paraphenylenediamine and methyl glyoxal by the extract; the inhibition of succinate, but not paraphenylenediamine, is partially removed ($\frac{1}{2}$ — $\frac{3}{4}$) by methylene blue. (Cyanide reacts with p-phenylenediamine.) 8. Cyanide increases the respiration of the extract itself, up to 100 per cent.

¹ Aided in part by a grant from the Otho S. A. Sprague Memorial Foundation.

9. A protein-lipoid fraction can be prepared from the extract by iso-electric precipitation at pH 4.6 which, after washing and resolution, still respire $\frac{1}{3}$ as much as the original extract. This preparation (developed by Dr. D. R. Briggs) can oxidize succinate and p-phenylenediamine almost as rapidly as the original extract. Analysis of these enzyme systems is being continued.

*The action of iodoacetate and glutathione on nerve and muscle.*¹ R. A. COHEN (by invitation), R. W. GERARD and N. TUPIKOW (by invitation). Department of Physiology of the University of Chicago.

Others' findings suggest that iodoacetate (*I*) acts by destroying the catalytically important glutathione (*G*) of cells. This is but partially true for frog nerve and muscle since *G* does not fully antagonize *I*; further, *G* has a toxic action.

M/1000 *I* and M/500 *G* were usually used; stronger and weaker solutions act similarly. Mixed *in vitro*, both substances become inactive, but not within 2 days. *I* cuts nerve respiration to one-fifth in 10 hours, *G* to about half, the mixture to three-fifths; *G* added 30 minutes after *I* is barely effective in reversing any *I* action. *G* prevents the phosphocreatin breakdown induced by *I* (3 hours); but not that of pyrophosphate. *I* reduces action potentials a third in 2 hours, *G* blocks in 20 minutes (not in a later series); *I* replacing *G* restores potentials one-third; a fresh mixture, even with excess *I*, always blocks.

I cuts muscle respiration to one-tenth in 6 hours; *G* depresses to half after an early increase. Added *G* largely prevents the *I* respiration decrease. *I* destroys two-thirds to three-fourths of the pyrophosphate and phosphocreatin (3 hours); *G* largely prevents this. Resting in *I*, an isolated sartorius shows marked rigor at three hours; in *I* and *G* it remains relaxed. Stimulated every ten seconds during alternate minutes, rigor begins in the *I* muscle in 10 minutes, in the *I* and *G* one in 15. In both, the shortening, after forty to sixty minutes, equals that of the normal twitch. Twitch height starts falling in *I* in 15 minutes and is zero in 26; with *G* present, in 16 and 31 minutes respectively. *G* alone has no effect (contrast nerve). Injected into a lymph sac of the intact frog, *G* does not influence muscle length or chemistry. *I* causes marked rigor, with parallel loss of pyrophosphate, in an hour; *G* with *I*, even in separate sacs, markedly retards chemical changes and rigor.

These results indicate that, beside specific actions on nerve and muscle, iodoacetate slowly destroys cell mechanisms independent of, as well as related to, glutathione.

The loss of sensitivity to gonadotropic hormones. J. B. COLLIP, H. SELYE (by invitation) and D. L. THOMSON. McGill University, Montreal.

Rats treated for a long period with the anterior-pituitary-like hormone (A.P.L.) of pregnancy urine lose their sensitivity to this substance. Their ovaries may even show signs of severe atrophy. The ovaries of such rats will still respond with rapid increase in size to the injection of gonadotropic extracts of pituitary origin. Similarly, rats which have lost their sensitivity to the gonad-stimulating principle of the hypophysis will still respond to A.P.L.

¹ Aided by grants from the National Research Council and the Ella Sachs Plotz Foundation.

*The relationship of contracture of skeletal muscle to serum calcium in cats.*¹

HELEN C. COOMBS and DONALD S. SEARLE (by invitation). Department of Physiology and Physiological Chemistry, New York Homeopathic Medical College, New York.

The effects of calcium deficiency on the nervous system have been extensively investigated. In these experiments, the object was to determine what changes, if any, take place in denervated skeletal muscle in response to electrical excitation, under conditions of known calcium deficiency.

Four groups of cats were studied. These were

1. Controls (10 cats).
2. Controls after continued bromide feeding (10 cats).
3. Untreated cats after thyroparathyroidectomy (10 cats), or parathyroidectomy (5 cats).
4. Bromide treated cats after thyroparathyroidectomy (10 cats).

The bromide treated cats were fed from ten days to two weeks before operation or experiment with one gram of sodium bromide daily. Aseptic removal of the thyroparathyroids or parathyroids only was done with the electric knife forty-eight hours before the acute experiment. Ionized calcium and inorganic phosphorus of the blood serum were determined at the beginning of each experiment.

Following the administration of urethane, curare was injected and artificial respiration maintained. The rectus abdominis muscle was freed to the lower border of the ribs, leaving the circulation intact, and the upper end was attached to a lever recording only in the vertical. Uniform stimulation at the rate of once a second was done with a weak induced current.

Results. 1. The controls uniformly presented the ordinary typical features of *treppe*, contracture and fatigue. The curves were not altered by intravenous injection of calcium gluconate.

2. Bromide controls presented the same features as normal controls.

3. Thyroparathyroidectomized and parathyroidectomized cats exhibited exaggerated contracture, which was greater, the greater the fall in calcium. On intravenous injection of calcium gluconate followed by a wait of half an hour, a fresh dissection on the opposite side, under stimulation, showed only the degree of contracture observed in control cats. *

4. In thyroparathyroidectomized cats after bromide feeding, tetany usually does not appear. Such animals, however, show almost the same exaggerated contracture as the animals in group 3.

The following table gives the averages for the different groups.

	AMOUNT OF CONTRACTURE	AVERAGE CALCIUM	Ca/P RATIO
		<i>mgm. per 100 cc. serum</i>	
Controls.....	*	10.0	1.9-2.0
Controls plus bromide.....	*	10.5	2.0
Uncomplicated thyroparathyroidectomy.....	****	5.4	0.9
Bromide plus thyroparathyroidectomy.....	***	7.8	1.5

So far as the extent of these experiments goes, the results in cats after thyroparathyroidectomy and parathyroidectomy appear to be identical,

¹ The cost of these investigations was defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

since in both cases, the magnitude of the calcium fall is in close correlation with the increased degree of contracture. Further evidence is needed, however, on the activities of other ions which are important in muscular reactions.

Effects of cautery of the brain on the fetal rat. E. L. COREY. Physiological Laboratory, University of Virginia Medical School.

Living fetuses recovered from the uterus 1, 3, 4, 5 and 6 days following electro-cautery in the region of the hemispheres, or those born alive or dead following such procedure, exhibited certain gross bodily abnormalities. There was commonly a flattened cranial vault, a malplacement of eyes and ears, an enlarged and protruding tongue, a thickened body outline with notably heavy neck and jaw, and a fore-shortened nasal and maxillary region. Little or no repair of the injured area was observed, although the general vascularity appeared unimpaired. Spontaneous movements and those elicited on tactile stimulation, e.g., by bristle or needle, showed furthermore no qualitative differences distinguishing them from those seen in litter-controls, although they appeared weaker and more sluggish. Stimuli normally initiating fetal movements do not therefore appear to originate within the cortex. It is suggested that the fetal cortex may nevertheless be necessary for quantitatively normal movement.

Of 70 operated fetuses, 47 survived until term or were recovered alive from the uterus at some time during gestation; the remainder were either aborted (1) or resorbed. Nine of those surviving until term were born alive and 1 dead; 6 were eaten (alive?) by the mother at birth, and 11 died *in utero* at term. None of the operated animals born alive survived over 24 hours. From these observations it is apparent that the decorticate or decerebrate rat fetus can survive until term, regardless of the stage of development (16 to 37 mm.) at which the operation of cerebral ablation is performed. It is also suggested that the early death of those fetuses which were born alive may be attributed to factors which are pre-natal in origin. This is indicated by the high mortality which occurred just before birth. When body weight was taken as a criterion, no retardation of fetal growth following cautery of the brain could be definitely demonstrated. To date a total of 95 experimental fetuses from 28 litters have been employed in these and various control experiments.

Studies of vitamin G (B_2) deficiency in dogs: preliminary communication.

GEORGE R. COWGILL, H. M. ZIMMERMAN and ETHEL BURACK (by invitation). Departments of Physiological Chemistry and Pathology, Yale University.

Two groups of dogs, eight and twelve in each group respectively, were fed "artificial" diets deficient only in vitamins G (B_2) and C (which the dog does not require in its diet). Food intake was maintained perfectly for 30-60 days after which a partial anorexia occurred resulting in varying degrees of general inanition. Eventually a state of collapse supervened. When intramuscular injection of a source of vitamin G (B_2) (Lilly Liver extract no. 343) was not delayed too long, striking recoveries were brought about within six hours. Subsequent administrations of subminimal doses in an endeavor to produce a state of chronic vitamin deficiency were partially successful, dogs being kept alive in this way for about a month after the onset of the initial collapse. The total periods of the experiments

ranged from five to seven months. Considerable loss of hair, vomiting, and diarrhea were of frequent but not invariable occurrence. The phenomena described as "black tongue" were *not* observed, although control animals fed the Goldberger diet no. 195 readily exhibited them.

The anatomic changes induced in our vitamin G (B_2) deficient animals may be summarized as involving primarily: marked demyelination of peripheral nerves, including the vagus, and degenerative changes in the posterior columns, the posterior nerve roots, and less often, the anterior roots of the spinal cord. These lesions occurred only in animals subsisting on the diet for at least five months. Six control dogs failed to show such lesions.

These changes in the central nervous system correspond to those frequently described in human pellagra. This finding suggests that vitamin G (B_2) deficiency is a factor contributing to the production of the syndrome of pellagra. Inasmuch as not a single animal developed "black tongue," lack of vitamin G (B_2) does not appear to be the primary cause of the stomatitis and glossitis characteristic of this canine disease.

Dextrose utilization in animals with Eck fistulae. LATHAN A. CRANDALL, JR., JOHN VANDOLAH (by invitation) and FREDERICK FITZ (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School.

In corroboration of a number of previous investigators, we have found that normal and Eck fistula dogs show similar blood sugar changes after the administration of dextrose by mouth. Oral sugar tolerance methods are too crude to demonstrate any change in tolerance. However, when dextrose is given intravenously at a constant rate for one hour, utilization by normal dogs varies between 1.8 and 1.9 gram per kilo while Eck fistula dogs use from 2.0 to 2.4. The results on our 10 normals coincide with those found by Cuthbert (unpublished) in more than 40 normal dogs; we have made 25 determinations in 14 Eck fistulae animals. The values for blood sugar during and after injection are comparable in normal and Eck fistulae dogs, showing that the increase in tolerance is not to be explained by a higher renal threshold.

The effect of 5 and 10 grams per kilo dextrose on metabolic rate and R.Q. were studied in 3 ducks after ligation of the portal vein. Preliminary results indicate that while it requires 10 grams of dextrose per kilo in the normal animal to bring the R.Q. above 1.0, 5 grams will suffice after portal ligation. In normal ducks the highest R.Q. after 5 grams per kilo was 0.95; in those with portal ligation the same amount usually caused a rise above 1.0, the highest value being 1.4.

Although the increase in dextrose tolerance exhibited by the Eck fistula dogs is slight, we believe that it is significant, since the values are higher than those obtained on any normal animals. The increase in carbohydrate tolerance sometimes noted in diabetes coincidentally with the onset of liver disease also suggests that liver dysfunction may be associated with an increased ability to utilize sugar. The results obtained readily explain the discouraging results which have attended the clinical application of liver function tests based on dextrose tolerance.

The effect of vitamin A deficiency on the salivary conditioned reflex induced by morphine. GEORGE CRISLER. The Department of Physiology, West Virginia University, Morgantown, West Virginia.

Salivary fistulae were prepared in dogs by exteriorizing the papilla of Wharton's duct. The animals were conditioned with morphine and then placed on specially prepared vitamin A free but otherwise adequate dog biscuits which were fortified with carotene during the control period. The biscuits were demonstrated to be vitamin A free by feeding experiments on rats. During the experimental period the carotene was withheld. The conditioned secretion was inhibited by the loss of vitamin A, but the unconditioned secretion was not influenced within the time limits of the experiments. The mechanisms involved are being investigated. The weights of the animals were constant within 1 kgm. The appetite and water consumption did not suffer during the experimental period.

Blood iodine after total thyroidectomy in man. GEORGE M. CURTIS and LOUIS E. BARRON (by invitation). Department of Surgery, Ohio State University.

Total thyroidectomy was accomplished in four patients. The indications for this were: 1, 2, hypertensive heart disease; 3, hypertension of unknown etiology, and 4, congestive heart failure due to arteriosclerotic heart disease. Adequate control periods were established prior to the thyroidectomies. Following total thyroidectomy two patients were observed in the hospital for approximately one month, and at frequent periods subsequently. The effect of total thyroidectomy upon the blood iodine, the urinary excretion of iodine, the blood pressure, and the basal metabolism were particularly noted. The normal blood iodine in man, determined by the modification of the von Fellenberg procedure which we are using, is about 12 gamma per cent (0.012 mgm. per cent). It was increased in these patients previous to hospitalization. Immediately following the total thyroidectomy there ensued a transient increase in the blood iodine. This subsided during the first twenty-four hours. It was accompanied by a striking postoperative increase in the urinary excretion of iodine. The blood iodine then gradually decreased. In one patient it fell to 1.4 gamma per cent. In one instance the decrease was transient and was followed by a return to normal in about six weeks. The increased postoperative excretion of iodine in the urine gradually returned to a low normal level. Daily records of the blood pressure were made during an adequate preoperative control period. Following total thyroidectomy there ensued a definite decrease in the systolic and diastolic pressures. These stabilized about one month after thyroidectomy. When physical activity was resumed, the blood pressure returned to the original level. During the control periods, determinations of the metabolic rate revealed normal values in three patients. In the fourth, the preoperative basal metabolic rate was plus 30, although there was no evidence of thyroid hyperfunction. Following total thyroidectomy the basal metabolic rate fell to the minus side in two of the patients. In the patient whose original basal metabolic rate was plus 30, the lowest level attained, ten weeks after the thyroidectomy, was plus 6. There was no evidence of hypoparathyroidism in the four patients.

A histological study of the action of estrin in terminating pregnancy. FRED E. D'AMOUR (by invitation). University of Denver.

Previous work reported by the author indicates that appropriate dosages of estrin in the early stages of gestation will invariably prevent or

terminate pregnancy and that simultaneous injection of other hormones, which, from theoretical considerations, might be expected to overcome the effect of estrin do not do so.

The present study is a histological investigation of the effects of estrin upon the uterus. Three series of animals were used, the first received 10 r.u. daily from the first to the tenth day; the second, 20 r.u. from the first to the fifth day; and the third 50 r.u. from the 10th to the 15th day. The animals were sacrificed the day following the last injection. Random sections were made of the uteri and stained with H. and E., and with Mallory's connective tissue stain. As controls, unbred animals not in estrus, unbred animals in estrus, 11 day pregnant and 15 day pregnant animals were used.

The results indicate, for the animals injected in the pre-implantation period, that the uterus shows a considerable degree of hyperplasia of the uterine mucous membrane, a considerable degree of fibrosis, usually glandular hyperplasia and an increased number of ciliated cells in the Fallopian tube. The fluid filling the uterus is thin and watery, contains less coagulable material than the normal pregnant uterus and resembles the fluid found during estrus.

In the post-implantation stage the effect of estrin is probably directly upon the fetus, the only change in the uterus being an absence of the edematous structure characteristic of normal pregnancy.

The vitamin B requirement of female albino rats for maintenance and growth.

MARGARET DANN (by invitation) and GEORGE R. COWGILL. Department of Physiological Chemistry, Yale University.

An attempt has been made to determine the amounts of vitamin B required by rats at various weight levels for normal growth, as contrasted with maintenance. The animals received a diet believed to be adequate in every food essential except vitamin B₁, and this factor was supplied in limited amounts in the form of a yeast concentrate, previously assayed by the pigeon technique of Block, Cowgill and Klotz (1932). The treatment of the animals differed from that usually employed in vitamin studies, in that the doses were graduated to meet the needs of each individual rat.

For the growth study, sufficient amounts of the vitamin B supplement, adjusted when needed by small increments, were administered to permit each rat to grow at a standard rate. An arbitrary standard curve was chosen, based on observations of Smith and Bing (1928) upon supposedly normal female rats of this colony. For the maintenance observations, the animals were allowed to grow while receiving a complete diet until one group of them reached a weight level of about 80 grams, another group 130 grams, and a third 180 grams. They were then deprived of the vitamin B supplement until cessation of growth indicated exhaustion of their store of this factor, which usually permitted an additional growth of 10 to 20 grams. After this, it was possible to adjust each rat's dose so that a weight level, constant within ± 4 grams, could be maintained for 3 to 4 weeks or longer.

The maintenance requirement of female rats was found to be directly proportional to the body weight. For rats from 90 to 240 grams, this was close to 0.8 pigeon unit (about 2.1 international units) per 100 grams body weight. The vitamin B requirement for standard growth, however, was more nearly proportional to the $5/3$ rd power of the weight than to any

other function, and appeared to be from 3 to 5 times as great as the maintenance need.

The relative significance of electrolyte concentration and tissue reaction in water metabolism. HARRY A. DAVIS (by invitation) and LESTER R. DRAGSTEDT. Department of Surgery of the University of Chicago.

The stomach of the dog was isolated from continuity with the digestive tract by the method of L. R. Dragstedt and Ellis (1929) leaving the vagus innervation intact. The continuous loss of gastric juice through such a preparation produced marked hypo-chloremia, alkalosis, and dehydration. A total permanent fistula of the pancreatic ducts was prepared by a method devised in this laboratory. The continuous loss of pancreatic juice produced severe acidosis and dehydration. The administration of NaCl solution was found to be effective in combating the dehydration in both instances whereas water or isotonic glucose solutions were not. In this study a comparison was made between the ability of the animals with dehydration and acidosis and those with dehydration and alkalosis to retain equal amounts of isotonic NaCl and glucose solutions. In general the animals with alkalosis retained the water better than those with acidosis.

The relation of auditory action potentials to the electrical response of the cochlea. H. DAVIS and A. J. DERBYSHIRE (by invitation). Harvard Medical School.

In the electrical response from the round window a component due to the action potential of the auditory nerve has been identified. If this is discounted the true cochlear response is found to conform very closely to the wave form of the stimulating sound modified by non-linear distortion. The threshold curve for this response in the cat is very nearly identical with the human audibility curve.

The latency of the action potentials in the auditory nerve is about 0.8 σ and diminishes slightly with increasing intensity of stimulation. Study of responses to unsymmetrical impulsive stimuli shows that the nerve impulses are initiated during the phase of increasing electrical positivity of the cochlear response at the round window.

The maximal frequency of nerve impulses in a single neurone is about 1,000 per second. At about this frequency the action potential wave falls to less than half its initial value during two seconds of stimulation, due undoubtedly to prolongation of the refractory period and consequent alternation of response. With lower frequencies the fall is less, with higher frequencies the first responses are already small. A similar maximum of "equilibration" occurs at a frequency of about 2,000 per second.

The relation of height of the initial action potential wave to strength of stimulus, in the frequency range where alternation is absent, is roughly hyperbolic and almost identical with the corresponding relation for the cochlear response. The threshold curves are also practically identical. We infer that in this frequency range and probably for all tones the sensitivity of the normal ear is determined primarily by the physical properties of the sense organ and that the sensation of loudness depends upon the total number of active nerve fibres and is independent of the frequencies of nerve impulses which they carry.

The physiological response to high concentrations of carbon dioxide and the nature of functional mechanisms involved. II. Alterations in respiratory activity. F. C. d'ELSEAUX and M. L. PETERMANN (by invitation). Harvard University.

Constant mixtures of carbon dioxide ranging from 5 per cent to 25 per cent were inhaled by adult human subjects. The changes in acid-base balance, in respiratory activity, in circulatory activity, and in oxygen transport were studied. The present report is confined to a discussion of the changes in respiratory activity.

The changes in pH and in oxygenation of arterial and of internal jugular venous blood were followed throughout the inhalation. The pH was gasometrically determined by use of observed carbon dioxide dissociation curves and the Henderson-Hasselbach equation. The data obtained are compared with similar data obtained during the inhalation of 4 per cent oxygen in nitrogen, during the ingestion of sodium bicarbonate, and during the ingestion of ammonium nitrate.

The nature of the response in each type of procedure is discussed.

The findings are interpreted in the light of those of other workers in an effort to understand the functional mechanisms of respiratory regulation.

The effect of barbiturates on the embryo and on pregnancy. JAMES M. DILLE (by invitation). Georgetown University, School of Medicine, Washington, D. C.

These experiments were performed on guinea pigs, rabbits, and cats. The types of experiments included the administration of a single large dose (from 225 mgm. to 300 mgm. of sodium barbital per kgm.), of repeated smaller doses (from 50 mgm. to 150 mgm. per kgm. of sodium barbital); and the study of effects of these doses upon the duration of pregnancy and upon the concentration of the drug in the placenta, amniotic fluid, and fetus.

The duration of pregnancy was usually shortened by the administration of a single large dose, and by oft-repeated smaller doses, although one rabbit showed normal delivery eight days following the administration of 250 mgm. of sodium barbital per kilogram, and the embryos showed no evidence of the presence of barbital. The concentration of barbital in the fetus was, in almost every case, considerably higher than that found in the uterus, placenta, amniotic fluid, and blood of the mother. Also the concentration of barbital in the embryo approached anesthetic levels, and in certain cases even exceeded this. The embryo of a rabbit which received 300 mgm. per kgm. of sodium barbital, contained 0.55 mgm. per gram, 15 minutes after the injection of the mother.

In acute experiments it was found that the barbital content in the embryos and amniotic fluid progressively decreased, while that of the placenta increased until about two and a half hours after injection.

Preliminary experiments indicated that not only barbital but also other barbiturates permeate the placenta and may be detected in the embryo.

Barbital was also detected in the milk.

On the excretion of xylose by man. R. DOMINGUEZ and E. POMERENE (by invitation). St. Luke's Hospital, Cleveland.

After 2 to 3 hours following the ingestion of xylose, the time change in the plasma concentration, x , in milligrams per 100 cc., and the minute rate

of excretion in the urine, y , in milligrams per minute, can be represented by exponential equations of the form

$$y = y_e + ae^{-\alpha t}, \quad x = x_e + be^{-\beta t},$$

where y_e and x_e are the endogenous blanks of the respective variables, a , b , α , and β , constants to be determined from the experiments, e is the base of natural logarithms and t the time in hours.

Six experiments were performed in two normal human beings, subjects E and D. In two experiments the urine curve only was determined. The constants ($\alpha \log e$) and ($\beta \log e$) calculated by standard methods are: ($\alpha \log e$), for subject E, 0.1656, 0.2340, and 0.2412, for subject D, 0.2297, 0.2754, and 0.2868; ($\beta \log e$), for subject E, 0.1660 and 0.2432, for subject D, 0.2546 and 0.2305.

The ratio (α/β) from simultaneous experiments is 0.9975, 0.9621, 0.9022, and 1.1948, mean 1.014. Therefore, the relation between the rate of excretion of ingested xylose and the corresponding plasma concentration is linear, that is,

$$(y - y_e)/(x - x_e) = (a/b) = \text{constant}.$$

The mean value of this constant, the *excretion constant* of xylose, is 0.77.

The excretion constant of creatinine on subject E is 1.82. But, the half period in the drop of plasma concentration is 2.390 hours for creatinine and 1.204 for xylose, whereas the percentage recovery from the urine, in a mean interval of 8.117 hours following ingestion, is about 62 for creatinine and 26 for xylose. It appears as if xylose is disposed of by means other than renal excretion above. The excretion constant is, therefore, of no nuclei in theories of renal function.

The experimental production of lymph edema and elephantiasis. CECIL K. DRINKER, MADELEINE E. FIELD and JOHN HOMANS (by invitation).
Department of Physiology, Harvard School of Public Health, Boston.

Permanent obstruction of the lymphatics draining the hind leg of the dog has been secured by repeated intralymphatic injections of crystalline silica and 2.5 per cent quinine hydrochloride. Elephantiasis is one of the consequences of lymphatic blockage and an extreme susceptibility to value in streptococcic infection is another. These points will be illustrated.

Studies in laminar corticology. J. G. DUSSER DE BARENNE. Laboratory of Neurophysiology, Yale University.

By the method of laminar thermocoagulation of the cerebral cortex it is possible to destroy, at will, any number of consecutive layers of nerve cells of the cortex, starting from the outer surface.

Some of the results obtained are:

1. Normal motor reactions can be elicited from the motor cortex (field 4 of Brodmann) on faradic stimulation after destruction of the 3 superficial layers. For $1\frac{1}{2}$ to 2 minutes after this lesion the threshold for such cortical reactions is augmented; after this period the threshold for unipolar faradic stimulation returns to its "normal" value.

2. Histological examination in these experiments has shown that the location of the motor foci on the cortical surface corresponds with the location of the large and giant pyramidal cells in the 4th layer.

The facts sub 1 and 2 indicate that the motor reactions obtained are due to the direct stimulation of the large and giant pyramidal cells of the 4th cortical layer.

3. The destruction of the 3 superficial layers of the precentral arm area (arm region of fields 4 and 6 of Brodmann) usually results in a very slight, transient paresis of the contralateral hand. After 3 to 17 hours all symptoms of motor impairment have disappeared. In some animals no symptoms of motor deficit appear at all after such lesion.

4. The destruction of the whole thickness of the cortex of the precentral arm area results in a paralysis of the contralateral arm, lasting for about 4 days. Then functional recovery becomes apparent and is complete in about 3 weeks.

The facts sub 3 and 4 show that the paralysis after lesions of the "motor" cortex is due to the damage or destruction of the nerve cells of the 2 inner layers. In combination with the facts sub 1 and 2 those sub 3 and 4 indicate that such paralysis is due to the damage or destruction of the large and giant pyramidal cells of the 4th layer.

Conditioned motor reflexes in cats. S. DWORKIN. McGill University.

Cats are easily trained to raise the lid of a specially built container when a piece of meat is placed within it. Following the Pavlov procedure, the unconditioned (meat) stimulus is preceded by a new (conditioned) stimulus, and soon this latter stimulus evokes a response. In this way, cats have been conditioned to various musical and other sounds, to light, and to tactile stimuli. The cat is well conditioned after 40 and 50 tests, but interval responses may persist for some weeks. The latent period of a response is proportional to the strength of stimulus, being 1 to 2 seconds for a strong sound and 2 to 4 seconds for a weak sound.

The development of inhibition has also been studied. Extinction and conditioned inhibition may be accomplished readily in the cat, there being little difference from the dog. Differentiation, however, between stimuli of like quality is more difficult to establish. Thus the capacity of the cat to discriminate pure musical notes is considerably less than that of the dog.

Long maintained generalization to musical sounds makes the cat a very suitable subject for hearing tests, and in most animals it has been possible to explore the hearing range in the space of 4 to 5 weeks.

The renal tubule of the frog and mammal as affected by mercury. J. GRAHAM EDWARDS. University of Buffalo.

The epithelium of the renal tubule in the kidney of the frog, rat, rabbit and guinea pig shows at death the following sites of injury when the animal has been given intraperitoneally 0.4 mgm. per 100 grams weight of a solution of HgCl_2 , 0.04 per cent: frog, the distal half of the proximal portion of the convoluted tubule; rat, the entire tubule except the neck and thin segments; rabbit, the entire tubule and duct system; guinea pig, the ascending limb of the medullary loop.

When these animals are given Ringer's solution, solutions of sodium ferrocyanide or sodium thiosulphate equivalent in concentration and volume to that of the injected HgCl_2 , or of greater concentration than the latter, the epithelium is better preserved although longevity is not markedly increased. In the kidney of the guinea pig given HgCl_2 and thio-

sulphate a much more marked injury is seen in the ascending limb of the tubule than when HgCl_2 is given alone. Rabbits given 4 mgm. of HgCl_2 every 48 hours for 96 hours and at the end of 288 hours survive longer than those given a single injection of 10 mgm. Those given a total of 16 and 21 mgm. of HgCl_2 and an equivalent amount of sodium ferrocyanide or thio-sulphate, respectively, lived for 133 days. The epithelium of the tubule was very much better preserved at death than that in the kidney of animals given a single dose of 10 mgm. of HgCl_2 and which died at the end of 7 days, although the glomeruli had, in the longer lived animals, undergone hyalinization and partial replacement by connective tissue thereby closing the lumen of the neck segment. The lesion in the kidney of these animals is definitely restricted to the lower half of the proximal convolution and the ascending limb of the medullary loop. Studies of human nephritic kidneys show segmental injury comparable with certain of those mentioned above.

The sites of injury were determined by a study of serially sectioned macerated tubules as well as by a study of serial sections prepared in the usual way. This method also makes possible the determination of the variation in tubule degeneration following various stages of glomerular degeneration with which such tubules are connected.

The oestrous cycle of the hairless rat. FREDERICK E. EMERY. University of Buffalo.

The factors that produce the hairless condition in these animals are unknown. It seems likely that the endocrine organs are not normal. The present report deals with the reproductive and pituitary glands. The ovaries and uterus are smaller and the pituitary is somewhat larger than those of the albino rat. Oestrus occurs infrequently and the dioestrus period is usually a month or more in length. Yet oestrus is readily produced by pituitary implants or extracts. Both males and females are fertile.

Manifestations of segments in the action potentials of myelinated axons. JOSEPH ERLANGER and E. A. BLAIR (by invitation). Department of Physiology, Washington University School of Medicine.

The record of the conducted monophasic axon potential, when the nerve is polarized anodally at the proximal (ground) lead, develops on its ascending limb two to four step-like discontinuities. The earliest of these steps is the lowest and the latest the highest. As the polarization increases the steps increase in height and separate until, at a critical strength, the latest of the steps, usually one, drops out. This happens when the start of the highest step has been pushed back until it rests on or slightly behind the crest remaining after the defection. In like manner each of the highest of the remaining steps can be eliminated from the picture in succession. Anodal polarization at the ground makes a diphasic potential monophasic when any negative element drops out; anodal polarization at the grid, typically, removes the elements, positive and negative, progressively from the latest to the earliest. With an interelectrode span of 6 mm. seven such steps have been demonstrated.

These results signify that the steps are made either by a steadily progressing process that can manifest itself only as it passes nodes and that anodal block develops at nodes, or by a process that progresses in jumps

from segment to segment and that blocking is effected by serial depression of the irritability of the segments. If the latter is the case restimulation might take place either in accordance with Lillie's concept of stimulation by eddy currents, or by means of a potential acting across effective transverse interfaces at the nodes.

Occasionally records of axon potentials initiated by stimulation through the ground lead with either ascending or descending currents are compounded of a very low and a later high step. This observation indicates that with either arrangement it is possible to stimulate a segment that contributes relatively little to the record.

The effect of the digestion of food on the blood flow in certain blood vessels of the dog. HIRAM E. ESSEX, JULIA F. HERRICK, F. C. MANN and EDWARD J. BALDES. Mayo Clinic and the Mayo Foundation, Rochester, Minnesota.

By the use of the thermo-stromuhr method of Rein the influence of digestion on the blood flow has been studied in the femoral, carotid, and mesenteric arteries. There is a marked prolonged increase in each of these vessels when digestion is in progress. The time of the onset and the duration of the increase is influenced by the character of the food but the magnitude is relatively the same regardless of the type of food ingested. In general the highest blood flow values obtained during digestion are about twice those obtained after an eighteen hour fast. The initial increase in blood flow following a semi-fluid meal rich in carbohydrate occurs in 10 to 20 minutes while as much as three hours may elapse before the blood flow begins to increase after the ingestion of a raw lean meat meal. The increased blood flow is accompanied by an increased pulse and respiratory rate. The increase in blood flow does not appear to be due to a reflex vasomotor mechanism since significant changes in limb volume and surface temperature were not observed and comparable increases occurred in the femoral artery of dogs that had previously had a lumbar sympathectomy performed. Significant changes in the blood volume were not apparent by the use of the dye method. The increased blood flow may be associated with a decreased circulation time. The increase in blood flow does not appear to be due to mechanical causes.

Studies on the injury potential of muscle. J. A. E. EYSTER and SARA L. HOOPES (by invitation). Department of Physiology, University of Wisconsin.

In complete fatigue of muscle induced by stimulation through its nerve or directly, there is no reduction of the injury potential, unless the stimulating current is excessive. Irritability may thus be lost without loss of injury potential, a condition previously shown to exist in nerve in chloroform and ether narcosis. Excessive direct stimulation of muscle causes a reduction of the injury potential to zero or may reverse it temporarily. This condition is associated with loss of irritability. Gradual recovery of injury potential and with it irritability may occur, especially in an oxygen rich atmosphere. These observations furnish additional evidence for the view that the condition in nerve or muscle making possible the development of an injury potential is an essential one for the presence of irritability, but that irritability may be lost through effects upon other factors, the tissue showing an injury potential of normal magnitude.

The electrical field around heart and skeletal muscle. J. A. E. EYSTER, FRANK MARESH (by invitation) and M. R. KRASNO (by invitation). Department of Physiology, University of Wisconsin. (Read by title.)

The tortoise heart or frog's gastrocnemius muscle was placed in the center of a circular disc 43 cm. in diameter containing a layer of Ringer's solution three millimeters in depth. Two electrodes were fixed at the margin of the disc at the two ends of the axis of minimal potential. A movable electrode was placed at various points at different distances and along different axes with reference to the preparation. Action potential curves were recorded by connecting through a two stage direct coupled amplifier to a string galvanometer. Single contractions of the skeletal muscle preparation were secured by stimulation through its motor nerve. The electrogram was recorded simultaneously by a second string galvanometer from electrodes leading directly from the preparation. The constant curve thus obtained served as a time reference to the potential curves recorded from distant points.

The action potential curves recorded along different axes around the heart showed a wave form progressing in time relative to the fixed electrogram direct from the heart. The potential field determined from these progressing wave forms by plotting equipotential points at a fixed time with reference to the electrogram is asymmetric with respect to the origin and departs from the symmetric field given by a single stationary dipole. The interpretation is that of a series of moving electrical charges with asynchronous time relations and along different axes in the heart muscle, which at no time fuse to represent a single equivalent dipole. The form of the field may be simulated by an asymmetric disposition of artificial dipoles. The gastrocnemius muscle shows a similar field but with less departure from that of a single dipole. The experiments are being continued with parallel fibered skeletal muscle.

The dark-field microscopy of blood platelets in clotting and citrated blood. J. H. FERGUSON. Laboratory of Physiology, Yale University. (Demonstration.)

The use of the dark-field microscope in the study of the rôle of platelet alterations in blood coagulation is demonstrated by a comparison of the data on clotting blood and on citrated plasma.

Gastric anacidity following pilocarpine injections in monkeys. J. H. FERGUSON and ELIZABETH R. B. SMITH (by invitation). Laboratory of Physiology, Yale University.

Controlled fractional gastric analyses have been made on a series of green monkeys (*Lasiopyga callithrix*, (Elliot) or *Cercopithecus aethiops sabaesus* (Schwarz)). Pilocarpine in doses exceeding a certain threshold produces a complete disappearance of the free gastric acidity in 10 to 20 minutes at whatever stage in the digestion of the meal the drug is exhibited. The approximate thresholds for this anacidity effect are (1) 0.2 mgm. per kilo by lateral cerebral ventricle; (2) 2.0 mgm. per kilo intravenously, and (3) 6.5 mgm. per kilo subcutaneously. The total acidity follows the free acid curve but the total chloride secretion is unaffected. The phenomenon is not a neutralization effect by *a*, food residues; *b*, mucus and swallowed saliva, or *c*, regurgitated bile. Antagonism by atropine indicates that it is

a parasympathetic effect. It can be antagonized only with difficulty by histamine.

The effects of partial and complete adrenalectomy on experimental diabetes.

H. WARD FERRILL (by invitation), J. M. ROGOFF, B. O. BARNES and V. B. SCOTT (by invitation). University of Chicago and Western Reserve University.

Using dogs as experimental animals, a quantitative study has been made of the glycosuria resulting from pancreatectomy. In some cases one adrenal was removed before the pancreas, while in other cases the pancreas was removed first and the one adrenal removed after the animal had been carefully standardized on a constant diet and insulin therapy. In one animal the adrenal on one side and half of the other gland was removed prior to pancreatectomy. The glycosuria was considerably less than normally observed after pancreatectomy, in those animals having a partial adrenalectomy 10 to 18 days before the pancreas was removed. In those cases where the pancreas was removed first, the insulin therapy could be diminished or discontinued after unilateral adrenalectomy, without severe glycosuria developing. These animals gradually lose weight and some have died after about 4 weeks. No accessory pancreatic tissue was found at autopsy. These studies are being continued and extended to animals with both adrenals removed.

The composition of lymph and edema fluid from regions with blocked lymph drainage. MADELEINE E. FIELD and CECIL K. DRINKER. Department of Physiology, Harvard School of Public Health, Boston.

As a result of lymphatic obstruction, removal of extravascular protein becomes difficult and perhaps impossible. During the past year we have had opportunity to collect and study lymph and edema fluid under circumstances of lymphatic obstruction, and an account of these observations throws light on the functions of the lymphatic system.

The relation of the adrenal cortical hormone to vitamins B(B₁), C and G(B₂).

W. M. FIROR and ARTHUR GROLLMAN. Department of Experimental Surgery and Department of Pharmacology and Experimental Therapeutics, School of Medicine, Johns Hopkins University.

Numerous recent publications have stressed a relationship between the activity of the adrenal cortical hormone and vitamins B and C. Our experiments have demonstrated the absence of any such interrelations. The apparent partial replaceability of vitamin C by adrenal cortical preparations reported by previous observers is due to the presence of ascorbic acid in such extracts. When this is removed the extract, whose potency is unaffected as regards its life-prolonging effects in adrenalectomized animals, shows no appreciable anti-scorbutic activity in guinea pigs. Crystalline vitamin C is found to be more effective when administered intraperitoneally than by mouth. It is thus fallacious to argue that an observed antiscorbutic activity following intraperitoneal injection of a given solution is not due to vitamin C if the same solution is less effective by mouth.

The adrenal cortical hormone had no demonstrable replacement activity in experimental avitaminosis B₁ or B₂ (G) of rats.

Ascorbic acid had no life prolonging action in adrenalectomized animals.

The effect of CO upon the embryonic fish heart. K. C. FISHER (by invitation) and LAURENCE IRVING. Department of Physiology, University of Toronto.

The heart of the embryo in the speckled trout egg is easily visible and the frequency of its beat is practically constant. Carbon monoxide (90 per cent) and O₂ (10 per cent) reduce the frequency. The reduction is gradual and requires from $\frac{1}{2}$ to 3 hours. Next follows a period of depression at around 50 per cent of the original frequency. The heart remains constant or declines in frequency only slowly thereafter. The inhibition is immediately removed by light, and in some cases completely. Dim light or darkness restores the inhibition promptly. The light effect may be repeated many times. The speed of onset of inhibition by CO resembles the slow development of inhibition by CN and reduced O₂ tension, but the effect of light and dark is contrasted by their immediate action. The results show that the heart frequency is maintained by a respiratory catalyst similar to Warburg's respiratory ferment. The observations differ from respiration studies by providing evidence on a complete physiological system. The main issue is whether the heart can be supported by an alternative metabolic process, by a reserve created by the oxidizing system, or whether the oxidation must directly and immediately support the heart beat.

A special micro-manipulator. G. W. FITZ. Peconic, Long Island, N. Y. (Demonstration.)

A special micro-manipulator for rapid operation in *a*, selection and distribution of bacteria and other unicellular organisms for pure culture; *b*, micro-chemical analyses down to particles of the order of 10⁻⁷ gram.

Effect of sympathetic stimulation on the paralytic submaxillary gland. A. J. FLEMING and F. C. MACINTOSH (by invitation). Department of Physiology, McGill University.

It was demonstrated in cats, in which one submaxillary gland had been denervated 10 to 30 days previously by section of the chorda tympani, that on sympathetic stimulation the threshold of excitation in the paralytic gland was much reduced. When both cervical sympathetic nerves were stimulated with currents of equal intensity and duration, the paralytic gland secreted much more copiously than the normal gland; the secretory after-effect was also greatly prolonged in the paralytic gland. Injection of adrenalin gave similar results. The effect of sympathetic stimulation was not antagonized by atropine, but was abolished by ergotoxine. Pilocarpine acted more strongly on the normal than on the denervated gland. After small doses of physostigmine sympathetic stimulation gave rise to a marked secretion from the paralytic gland, followed after a definite latent period by secretion from the normal gland. It was concluded that section of the preganglionic parasympathetic fibres to the submaxillary gland heightens its excitability for sympathetic stimulation.

A study of the effect of temperature and of strength of stimuli on contractility in the plant mimosa. H. J. FULLER (by invitation) and W. E. BURGE. Departments of Botany and of Physiology, University of Illinois, Urbana. (Read by title.)

A leaf of the plant *Mimosa pudica* was stimulated by dropping weights

varying from 10 to 400 mgm. through a distance of 20 cm. and striking the plant. The extent of the bending of the leaf brought about by the stimulation was read off directly on a millimeter scale placed beside the plant. Four different plants were used and several determinations were made on each plant.

It was found that stimulating the plant by dropping the lighter weights, 10 and 20 mgm. for example, produced no bending of the leaf. However, when a weight was used that was sufficient to stimulate the plant, a maximal effect or bending of the leaf was produced and the use of heavier weights produced no greater effect. Hence it would seem that the plant *Mimosa*, like cardiac muscle, is apparently "all or none" in its reaction to different strengths of stimuli. The ease with which the plant fatigues and the long refractory period enter as complicating factors in establishing the "all or none" principle for the plant.

The effect of temperature was also studied. It was found that the plant was non-irritable at 10°C. At 20°C. stimulation was effective and produced a slight bending of the leaf; at 25°C. the stimulus was most effective, producing the most extensive bending, while at 30°C. the bending was not so great as at 25°C. but more than at 20°C. From the preceding it would seem that temperature affects contractility of the plant *Mimosa* very much as it does animal muscle.

Autonomic representation in the cerebral cortex. J. F. FULTON, MARGARET A. KENNARD (by invitation) and J. W. WATTS (by invitation). Laboratory of Physiology, Yale University.

Removal of the cerebral hemispheres of any higher vertebrate causes increased activity of certain viscera such as heart and gut and other organs controlled by the autonomic nervous system. This indicates that the cerebral cortex normally plays a part in regulating autonomic function, but it gives no clue as to what region of the cortex is involved. Stimulation and extirpation of various parts of the hemispheres of monkeys and chimpanzees have provided evidence that motor representation of the autonomic system lies in the premotor area, chiefly in its rostral portion (area 6a β of the Vogts). Stimulation of this region gives rise to vigorous peristaltic movements of the gut; bilateral extirpation also causes excessive motor activity of the gastro-intestinal tract and this undoubtedly accounts for the state of morbid hunger exhibited by these animals and for the obstructive intussusceptions which they sometimes develop.

Unilateral extirpation of the premotor area (and of no other region of the cortex) also causes marked vasomotor disturbance on the opposite side of the body, characterized by a failure of the reflex vasodilator mechanism. Thus when the temperature of the surroundings is elevated the temperature of the affected side of the animal's body rises much more slowly than that of the normal side. Reflex vasoconstriction in response to cold is not affected by the cortical lesion (Kennard, *Science*, 1934, 79). Sudomotor and probably pilomotor activities are also affected by premotor lesions. Other autonomic functions have still to be studied.

The effect of posterior pituitary preparations on the blood flow of the normal intact dog. E. M. K. GEILING, J. F. HERRICK and HIRAM E. ESSEX.
The Department of Pharmacology and Experimental Therapeutics,

Johns Hopkins School of Medicine, Baltimore, Maryland, and Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota.

With the Thermo-Stromuhr method of Rein as improved by Herrick, Essex and Baldes, blood flow measurements were carried out on resting, normal dogs after injection of various posterior pituitary preparations (the pressor and oxytocic fractions of Stehle and of Parke, Davis & Company—pitressin and pitocin). The following results were obtained. The blood flow in the femoral artery, femoral vein, carotid artery, and jugular vein shows a marked decrease following the intravenous injection of the pressor fraction of the posterior lobe of the pituitary gland. This decrease is prolonged and the pre-injection level is not attained until about one and a half hours have elapsed. When pitressin is inactivated with alkali, no effect on the blood flow is observed. The oxytocic fraction also has no significant effect.

The pressor fraction produces similar effects on the blood flow in the femoral artery of a sympathectomized and an adrenalectomized dog.

The effect of exercise on the acetone bodies in the blood of man on low carbohydrate diet. C. L. GEMMILL. Department of Physiology, School of Medicine, Johns Hopkins University.

A study was made of the total acetone bodies in the blood of three subjects before and after exercise with the subjects on normal and low carbohydrate diets. No significant changes were observed following exercise with the subjects on normal diets. On low carbohydrate diets, with a general elevation of the acetone bodies, a further increase was found after work. The maximal rise in each case was observed two hours after exercise. These experiments suggest that fat may be used as such to supply the energy for muscular contraction in man.

*Brain action potentials.*¹ R. W. GERARD, W. H. MARSHALL (by invitation) and L. J. SAUL. Department of Physiology of the University of Chicago.

The electrical exploration of the brain, reported earlier (Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1123), has been extended with the aid of a Horsley-Clark stereotaxic instrument, permitting accurate anatomic localization. Eyoked activities, by light, sound and touch, and those "spontaneously" present were picked up by a concentric needle electrode, amplified and fed into a loud speaker and a cathode ray oscillograph. Records obtained with the latter will be demonstrated.

Auditory responses were accurately located in the root of the acoustic nerve, accessory nucleus of the nerve, trapezoid body, lateral lemniscus, medial geniculate body, and auditory radiations. An active fibre bundle is present ventrolateral to the cornu ammonis at the level of the geniculate; and more caudal, activity was obtained from Forel's decussation and more lateral structures. Activity was greatest where the lateral lemniscus entered the inferior colliculus and from the nucleus of the colliculus.

Optic responses were strongest in the optic tract before entering the lateral geniculate body, less in the cell mass itself and quite strong in the optic radiations. These were easily traced through the white matter and

¹ Aided by a grant from the National Research Council.

responses obtained from the cortical area striata. An important bundle passes over the lateral surface of the cornu ammonis, as stated by Poljak on histological evidence. Powerful responses were also obtained from the superior colliculus; and optic stimulation acts upon auditory responses at the junctions of the colliculi and of the geniculate bodies. Probably in the lateral geniculate, a double rhythm is imposed on the optic paths, giving a total frequency of four a second. This is abolished by optic stimulation, as units get out of synchrony, to return after light is discontinued.

The background activity is marked and usually irregular in cortical radiations; very strong with sudden bursts in the cerebral peduncles; active in the central grey, and other nuclei, as ruber, lateral thalamic, etc. Bursts synchronous with respiration were obtained from the medial lemniscus. Localized cutaneous and proprioceptive responses appeared in the lateral thalamic nuclei.

With Doctors Case and Bailey collaborating, responses to auditory, optic and proprioceptive stimuli have been obtained from the exposed brains of patients.

Is breathing fundamentally a reflex phenomenon? ROBERT GESELL and CARL MOYER (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Whether the rhythmic discharge of the respiratory center is a localized physico-chemical process modified by afferent impulses or a reflex phenomenon modified by chemical conditions is considered.

Effects of varied combinations of afferent inflow suggest that breathing may be largely a resultant of numerous and various afferent nerve impulses.

The effectiveness of afferent impulses is profoundly influenced by chemical changes capable of modifying breathing. Results indicate depression of excitatory and inhibitory respiratory reflexes by CO_2 saturation and augmentation by CO_2 depletion and by anoxemia. These findings are tentatively employed to analyse respiratory phenomena.

The indirect proportionality of rhythm to intrapulmonary pressure is a reflex effect of changing lung volume. Assuming only a single inhibitory vagal reflex, augmentation of this reflex by sodium carbonate should slow the rhythm, and depression by CO_2 should accelerate it, at any pulmonary volume. Graded volume change with a dual vagal reflex control should at some point reverse the effects of both CO_3 and CO_2 . Experimentally CO_3 retards during inflation, accelerates during deflation and reduces amplitude at neutral volume. CO_2 acceleration during inflation is reversed to retardation during deflation. Lung volume, therefore, importantly determines the mode of response to chemical influences by changing the relative dominance of individual pulmonary reflexes.

Carbonate apnea is not due to lost excitability, but rather to augmentation of the vagal stretch reflex, for saphenous stimulation during apnea produces super-excessive breathing. CO_2 produces its relatively slower breathing by depressing vagal accelerating function by chemical vagotomy. Anoxemia produces its relatively rapid breathing by augmenting this function.

While the present experiments show the great importance of reflexes and their modification through chemical changes they are not necessarily opposed to an automatically discharging center under the influence of chemical and afferent nerve impulse changes.

It is suggested that the control of heart rate and blood vessel caliber may operate on the same principle as that of respiration.

Factors determining junctional block in hearts and heart strips. A. S. GILSON. Department of Physiology, Washington University School of Medicine, St. Louis.

Experiments upon the spontaneously beating and the driven heart under the effects of vagus stimulation and upon heart strips across which a compressing clamp has been placed yield qualitatively identical results with respect to the development of junctional block. It is concluded that three major factors determine the production of block. These are 1, the degree of "junctional resistance" which is essentially constant in a normal preparation with or without vagus stimulation. It increases with trauma, etc. 2. The "exciting value" of the impulse to be transmitted across the junction. This value is, in an intact, uninjured heart much greater than is necessary to give conduction across any of the junctions at the normal heart rate. 3. The excitability of the tissue on the distal side of the junction. In a normal tissue this is a function merely of recovery from refractoriness.

The second and third elements may be differentiated experimentally by the fact that an impulse may be blocked at a junction either because a normal impulse arrives at the junction during a period of refractoriness of the distal segment or because a premature impulse arrives at the junction and fails to activate the distal segment even though the latter has been allowed adequate time for recovery to a normal degree of excitability.

The interpretation of electrograms obtained from heart strips in a conducting medium. A. S. GILSON and G. H. BISHOP. Department of Physiology and the Oscar Johnson Institute, Washington University School of Medicine, St. Louis. (Read by title.)

The erroneous assumption has been made (Wilson, Macleod, and Barker, 1933) that when a heart is exposed in the body, one lead electrode (C) being in contact with the heart and the other a remote electrode (R) attached to a leg, the recorded electrical changes occur only under C, while R remains at an essentially constant zero voltage. The origin of this error is evidently assignable to an analogy with the condition that when two negligibly small poles of a doublet are considered as oppositely and equally charged, one positive and the other equally negative, a function of their mean voltage acts upon a remote electrode. This is therefore at zero potential. In the heart this condition does not hold. If it did, it would be difficult to account for the actuality of the clinical electrocardiogram.

For the case of an immersed heart strip at rest, injury effects will give some negativity at a remote lead, R. Consider this potential to be compensated by the usual devices. Suppose now that the strip be stimulated at a point as far as possible from C. R, being acted upon by the mean voltage from the surface of the strip will begin to show negativity which will increase as the active area spreads over the surface of the strip. C, being in contact with resting (positive) heart tissue will remain relatively

unchanged. When the wave of excitation encroaches on and develops in the region under C, there will be a swing of the galvanometer due to beginning and growth of actual negativity under C. As the wave of excitation passes on beyond C, a greater surface becomes active and the galvanometer shows further increase in negativity at R. If C be placed just beyond the end of the strip and R several centimeters farther away, the record may show no diphasicity of the R-complex. Qualitatively similar though quantitatively different changes during the recovery process will produce the T-complex.

The above facts are in harmony with a core-conductor hypothesis of tissue activity and they are in accordance with the laws governing the distribution of currents in a conducting medium.

The measurement of mitogenetic radiation from living organisms. OTTO GLASSER (by invitation). Cleveland Clinic Foundation, Cleveland, Ohio.

Attempts to measure Gurwitsch or mitogenetic radiations are being made with three different methods:

1. Rather dense yeast suspensions are placed in two tubes—one made of quartz and the other (control) of glass—and are exposed to "mitogenetic radiators" for the duration of from 1 minute to 1 hour. Then 1 cc. of each yeast suspension is added to 5 cc. of a peptone glucose medium and permitted to grow for twenty hours at a constant temperature. At the end of this period the yeast is killed with sulphuric acid and the radiated (quartz) and control (glass) suspensions are placed in hematocrit tubes and centrifuged. The more rapid growth of the irradiated suspension is indicated by a longer column of yeast particles in the tube of the radiated suspension as compared with the control suspension.

2. Yeast suspensions in quartz and glass vessels are irradiated with "mitogenetic radiators" for two hours. The increased turbidity of the irradiated solution (as compared with the control solution) is measured either by its increased absorption of a light beam or by its increased scattering of a light beam by means of a nephelometer using a zero method with two sensitive photoelectric cells and an amplifier system.

3. The radiation from "mitogenetic radiators" is measured by directing the radiation through a quartz window to a platinum plate which acts as the photoelectric material in a photoelectric cell and is connected with a Geiger counter. This combination is extremely sensitive and makes it possible to measure very slight radiations of ultraviolet light with extreme sensitivity. Readings taken when a thin glass plate is placed between the counter and the radiator furnish the control count. Discharges in the Geiger electron counter are amplified by means of a two-stage amplifier and can be heard on a loud speaker and registered on a tape recorder or a magnetic counter.

A study of ketosis in the rat. WALTER GOLDFARB (by invitation), SAMUEL BARKER (by invitation) and HAROLD E. HIMWICH. Department of Physiology, Yale University.

The absence of ketosis in the fasting or fat-fed rat has been used as evidence that the rat can oxidize the ketone acids without the simultaneous oxidation of antiketogenic substances. In the present experiments on phlorhizinized, fasted and fat-fed rats the foodstuffs metabolized have been determined from the oxygen consumption, respiratory quotient, and urinary nitrogen. Assuming a ketogenic-antiketogenic ratio of 2:1 we have

calculated the amounts of acetone that should be excreted and compared them to the observed yields. In the phlorhizin experiments the theoretical yields approximated the observed yields, and the discrepancies could be accounted for by errors in the methods used. The fasting and fat-fed rats excreted almost no acetone bodies in a large majority of the experiments. The non-protein respiratory quotients in most of the experiments indicated that a mixture of fat and small amounts of carbohydrate were oxidized. If these small amounts of carbohydrate were neglected, the theoretical yields of acetone were found to exceed the observed yields in 32 of 37 experiments. However, when the amounts of carbohydrate oxidized were included in the calculations, the theoretical yields were zero (as were the observed yields) in 67 per cent of the experiments. The amounts of acetone expected in the remaining experiments could be accounted for by the errors in the methods used. It is therefore probable that the rat requires the oxidation of a fairly definite proportion of anti-ketogenic substances when acetone bodies are oxidized.

Effect of oxygen absorbed through the skin upon the vascular reaction to stasis and to histamine. SAMUEL GOLDSCHMIDT and BARTGIS MCGLONE.

The University of Pennsylvania, School of Medicine.

The forearm, with arrested circulation, in an oxygen atmosphere for an appropriate time, fails to exhibit a reactive hyperemia upon reestablishing the circulation; additional evidence of the penetration of the oxygen into the skin.

Sir Thomas Lewis has concluded that the vasodilatation responsible for reactive hyperemia is due to an "H" substance, identical with histamine.

If so, it must be concluded that, in our experiments in the presence of oxygen, the histamine is 1, not produced, or 2, if present it is in an inactive form, or 3, is destroyed by the oxygen.

Lewis postulated that the "H" substance is a normal skin constituent which accumulates in sufficient amount, when the circulation is arrested, to cause vasodilatation.

The first two possibilities cited above would be excluded by this concept. If the histamine is destroyed by oxygen (the third possibility), one would expect to find either a diminution or a failure of the usual intradermal histamine reaction on the skin of an arm, with or without an arrested circulation, in oxygen. On the contrary, the reaction is obtained in oxygen with as high a dilution of histamine as upon the skin of a control arm in nitrogen.

Likewise the reaction to a scratch, which is alleged to be due to histamine released in the skin, is not visibly affected by oxygen.

The histamine reaction is rendered especially visible on the skin of an arm with arrested circulation in oxygen, since it stands out against a background of normal skin color.

For this reason one observes an unmistakable "flare," a finding incompatible with Lewis' belief that the histamine flare is *exclusively* a reflex arteriolar dilatation. The capillaries must of necessity undergo an active dilatation.

The effect of the development of the supernormal period on the relatively refractory period of isolated frog nerve. HELEN TREDWAY GRAHAM. Department of Pharmacology, Washington University, School of Medicine, St. Louis.

In a *freshly* isolated frog nerve, normal irritability is recovered 7 to 10 σ after a response (25–29°C.); following this relatively refractory period, the period of supernormal irritability is absent or insignificant. If the nerve is left in neutral Ringer's solution or in a moist chamber, and particularly if it is experimented upon, supernormality develops and the relatively refractory period is shortened to 1.5 or 2.5 σ ; the absolutely refractory period however remains constant. If the recovery of height of action potential after a previous response is observed at different times after the isolation of the nerve from the body, it will also be found to require a longer interval after the response in fresh nerve. Normal height of action potential is always regained after the same interval as normal irritability, this interval (the relatively refractory period) becoming shorter and shorter as the experiment progresses. These progressive changes are complete in $\frac{1}{2}$ to 1 $\frac{1}{2}$ hour, and thereafter the recovery curves and the supernormality remain the same for some time.

When time has been allowed for the development of supernormality, maximum irritability is attained after an interval equal in duration to the relatively refractory period in fresh nerve. The resting level of irritability and the normal height of action potential are constant throughout the development of supernormality and the accompanying changes in the recovery curves. After these changes have occurred, therefore, the irritability rises faster than before, reaching a higher level in the same interval, and the height also rises faster, reaching the same level in a shorter interval. The underlying change must represent an actual modification of the recovery processes.

In a nerve veratrinized after the relatively refractory period has become stationary, the degree of supernormality is increased chiefly through modification of the supernormal part of the rising irritability curve, so that the relatively refractory period is shortened very little more. The maximum irritability is still attained after an interval equal to the relatively refractory period in fresh nerve, though in the veratrinized nerve the delay in the subsequent fall of irritability prolongs the time during which the irritability seems to be practically maximal.

Modifications of coronary flow during aortic insufficiency, independent of changes in mean aortic pressures. HAROLD D. GREEN (by invitation).

Western Reserve University, Medical School.

Utilizing the method for measuring coronary inflow demonstrated with Gregg at these meetings, the effect of experimental aortic insufficiency on mean coronary flow was studied. The coronary rates of flow at perfusion pressures ranging somewhat above and below mean aortic pressures were measured *a*, under normal conditions of valve action; *b*, during temporary aortic insufficiencies of different degrees, and *c*, again under control conditions when valve action had been restored to normal. In favorable animals such a series of tests could be repeated quite a number of times.

Among the results obtained was the observation that the rate of coronary flow decreases definitely during the period of insufficiency, despite the fact that perfusion pressures were independent of aortic pressures and that the latter were kept constant. It therefore is probable, if not quite certain, that the altered dynamics of ventricular ejection and filling reduces coronary flow during an aortic lesion, independently of effects due to alterations of aortic pressure.

On the vagus as a pathway of efferent coronary constrictor nerves. CHARLES W. GREENE. Department of Physiology, University of Missouri.

At the last annual meeting evidence was presented for efferent constrictor nerve pathways by routes other than, or in addition to the vagus, probably via the stellate ganglion and the cardiac plexus.

During the current year surgical resections have been made of the roots of the vagi between the ganglion nodosum and the brain. After full degeneration the nerves were tested for functional reactions by stimulating the degenerated cervical vago-sympathetic trunks. The results of the procedure have shown a striking absence of all coronary constriction after degeneration.

An extra dividend in the experiments has been conclusive evidence of the presence of coronary dilators in the cervical vago-sympathetic after vagal degeneration. The dilators undoubtedly represent pre-post ganglionic pathways via the superior cervical ganglia. After vagus degeneration in the dog there is uncomplicated and pronounced coronary dilatation upon stimulating the inferior cervical ganglion.

We have secured no further evidence concerning the presence or absence of coronary constrictors in thoracic pathways.

The phasic variations in coronary flow, studied by an autoperfusion method.

D. E. GREGG. Western Reserve University, Medical School.

The systolic and diastolic variations in the rate of flow in the *ramus descendens anterior* were studied by the improved autoperfusion method demonstrated at these meetings. The use of the animal's own blood in place of Locke's solution may be expected to approximate more nearly normal conditions as regards temperature, chemical composition, viscosity and hence probably as regards the normal state of the vessels.

Simultaneous optical records of the fluctuating decline of pressure in the perfusion manometer together with pressure records from the aorta or left ventricle were taken. Beats in which the perfusion pressure levels compared reasonably to existing aortic pressures were selected for study.

Normal records show the following characteristics: During isometric contraction, the coronary flow decreases abruptly to a negligible amount or it may even exhibit a momentary slight backflow. The flow is practically stopped to about the end of reduced ejection, sometimes increasing slightly toward the end of systole. The rate of flow accelerates slightly as intraventricular pressure drops during isometric relaxation and the maximum rate of onflow is not reestablished until after this event.

The results more nearly agree with the interpretations of Anrep and his associates than with those recently reported by Wiggers and Cotton, from which they differ in 1, that at comparable aortic and perfusion pressures, systolic flow is a negligible quantity, and 2, that the resumption of diastolic flow is gradual, not abrupt. The cause of these discrepancies is being studied.

Demonstrations of phasic variations of coronary flow by an autoperfusion method and of the effects of aortic insufficiency on mean coronary flow.

DONALD GREGG and HAROLD GREEN (by invitation). Western Reserve University Medical School.

The optical method for studying phasic variations in coronary flow (Cotton and Wiggers) has been improved in the following particulars:

1. The animal's own blood (heparinized) was substituted for Locke's solution as a perfusion fluid for the anterior coronary artery. 2. A metal syringe was used as the compression chamber to create the driving force for coronary perfusion under a declining pressure. 3. During an experiment blood from the dog's right carotid was delivered by a shunt tube into the coronary artery. When a record was desired a portion of the carotid blood was diverted into the driving reservoir, placed under pressure and then by a second stopcock the shunt circuit was shut off and the reservoir connected to the coronary artery.

A modification of the method of testing the mean rate of coronary inflow, when saline solutions are employed, was also demonstrated. A large bore mercury manometer with a float serves as a recorder for both volume flow and coronary pressure at any instant. Simultaneous registration of mean carotid pressure readily allows an evaluation of mean flow at corresponding perfusion pressures. The use of the method to study the effects of aortic insufficiency was demonstrated.

Blood sugar changes during sixty-hour fasts. ESTHER M. GREISHEIMER.
The University of Minnesota.

The decrease in blood sugar during fasts of sixty hours has been determined in a group of eleven adults (7 women and 4 men), who were either students or instructors. Water, black coffee, and clear tea were allowed. Each subject did his usual work during the fast.

The blood was drawn after twelve, thirty-six, and sixty hours of fasting. The sugar was determined (in duplicate) by the Shaffer-Somogyi method. The following values were found:

	AFTER 12 HOURS	AFTER 36 HOURS	AFTER 60 HOURS
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
7 women.....	84.31	66.07	58.58
4 men.....	86.09	74.15	64.42
Entire group.....	84.96	69.01	60.70

The women showed a decrease of 30.52 per cent (25.73 mgm.) and the men a decrease of 25.17 per cent (21.67 mgm.). The women showed a fall of 21.63 per cent during the first thirty-six hours, and the men a fall of 13.87 per cent during this time. In this group, therefore, the women showed 70.89 per cent of the total fall, and the men 55.10 per cent, during the first thirty-six hours. It seems, then, that the fall is more rapid in women during the early stages of the fast.

Four women and one man showed acetonuria after thirty-six hours, and all the subjects except one woman showed acetonuria after sixty hours of fasting.

The weight loss varied from four to ten pounds during the fasting period.

Indications of an antagonism by certain substances to the differentiation factor in thyroxin. F. GUDERNATSCH and O. HOFFMAN (by invitation). New York University, Washington Square College, New York City. (Read by title.)

In amino acid experiments previously reported, we observed a physiological antagonism between arginine (growth factor) and thyroxin (differentiation factor). Further investigation of this antagonism included ornithine and guanidine, structurally related to arginine, and urea. Amphibian tadpoles were treated with these substances by immersion (1:1000 solutions) or repeated injections (1, 5 or 10 mgm). After several weeks, they received thyroxin, resp. diiodotyrosine. No other N was supplied in the diet. In some control sets, alanine or cysteine provided a check on N influence.

Immersion: *Rana sphenoccephala*. With arginine, onset and progress of thyroxin effect were definitely retarded, the life period was longer than in thyroxin alone. The ornithine group showed greater response to thyroxin than arginine, in time and extent, almost equaling the thyroxin control. Alanine paralleled, cysteine (growth factor) fell slightly behind control.—*Rana sylvatica*. Alanine and cysteine were somewhat behind diiodotyrosine, arginine more; guanidine slightly in advance at first, later somewhat behind; urea at first equaled, later ran ahead of diiodotyrosine. Alanine equaled thyroxin, cysteine kept somewhat behind, arginine more, guanidine slightly. Urea was the first to show initial thyroxin changes, later it paralleled thyroxin. In a *Bufo* experiment, ornithine retarded the thyroxin and diiodotyrosine effects.

The retarding arginine effect, varying in intensity, was observed in five additional experiments, including *Rana catesbiana* (young).

Injection, three experiments: *Rana catesbiana* (old). 1. In arginine-thyroxin, metamorphosis onset occurred definitely behind thyroxin, the effect appearing gradually; later somatic changes lagged behind control, except the very last. In ornithine there was more tail resorption, other changes about paralleled the control, with some specimens ahead. In urea, no retardation, even slight advance. 2. In arginine-thyroxin, onset slightly, later effects markedly behind thyroxin; in this set occurred the latest metamorphosis, death. Urea-thyroxin group was much ahead of arginine, slightly behind thyroxin. 3. Metamorphosis changes appeared as follows: thyroxin about equaled arginine 5 mgm., ahead of ornithine 5 mgm., ahead of arginine 10 mgm.

In immersion experiments, arginine gave definite, ornithine slight indications of delaying the thyroxin effect, guanidine was indefinite; urea had no such effect. By injection, arginine also delayed; ornithine and urea were indefinite, perhaps even making the organism more susceptible to thyroxin.

The respiratory quotient as a guide to mealtime intervals. HOWARD W. HAGGARD and LEON A. GREENBERG. Laboratory of Applied Physiology, Yale University. (Read by title.)

A method for rapidly determining the respiratory quotient has been developed. With its use the respiratory quotient at hourly intervals was determined on 400 normal subjects eating meals at various intervals. A definite correlation was found between the appearance of hunger and the level of the R.Q. On a number of subjects the blood sugar was also determined, and its variations were found to correspond closely with those of the R.Q. and with hunger.

An improved model of the hypodermic manometer and some tracings illustrating physiological applications and damping with viscous fluids. W. F. HAMILTON, GEORGE BREWER (by invitation) and IRVING BROTMAN (by invitation). George Washington University. (Demonstration.)

Certain recently described changes in the manometer demonstrated at the last meeting of this society are shown. These consist of modifications in the method of attaching the mirrors, the flexible membrane and the lead tube. It has been made much more convenient to control the direction of the light beam and to enter the vascular system with a cannula (Luer needle) on the end of a long flexible lead tube. It has been possible to keep the frequency of the instrument about 200 cycles per second and by filling it with fluids of increased viscosity it has been rendered practically aperiodic without appreciably slowing its time of response. Records are shown which illustrate the use of the manometer.

Circulatory responses to acetylcholine in normal dogs and in dogs with experimental aortic regurgitation with a preliminary note on the pericardium.

W. F. HAMILTON, IRVING BROTMAN (by invitation) and GEORGE BREWER (by invitation). George Washington University.

The fact that effective doses of acetylcholine produce smaller blood pressure effects upon unanesthetized man than they do in the anesthetized dog is probably due to the freer play of compensatory mechanisms in the unanesthetized organism. To compensate for the vasodilatation produced by acetylcholine one would expect to find a marked increase in cardiac output. This is denied in man by Weiss who has used the acetylene method of Grollman and by Starr et al. who administered the acetylcholine by mouth. Since the method of Grollman is not well adapted to follow marked increases in the output when these are accompanied by a marked shortening of the circulation time, we have decided to present data concerning the cardiovascular responses of the unanesthetized dog to acetylcholine. Our results are as follows:

In the unanesthetized normal dog 2 mgm. of acetyl-beta-methylcholine chloride produces a slight fall of blood pressure, a marked reflex cardio-acceleration, a decided quickening of the velocity of blood flow and a large increase in the volume flow.

In dogs with chronic experimental aortic regurgitation, the cardio-acceleration does not usually occur and the increased volume flow is produced by an increased stroke volume.

The fact that the total circulation time is reduced to a half or a third normal in the unanesthetized dog renders doubtful the applicability of the techniques previously used in studying the human cardiac output under acetylcholine and explains why such work indicates that the drug produces no change in the cardiac output even while producing marked symptomatic changes.

Two of us with the help of Miss Leah MacArthur have performed preliminary experiments in which the pericardium was opened and the dog allowed to recover. When one leaflet of the aortic valve was ruptured the animal died of acute pulmonary congestion and right ventricular dilatation. These sequelae do not ordinarily occur when the pericardium is intact.

Liver treatment in experimentally produced polycythemia. LOUISE HANSON (by invitation) and EUGENE DE SAVITSCH (by invitation). Physiological Laboratory of the University of Chicago.

In order to determine whether the liver may exercise a regulatory control upon the erythrocyte count not only when this is lowered, as in anemia, but also when it is high, as in polycythemia vera, a number of white rats with experimentally produced polycythemia vera were treated with intramuscular injections of a potent liver extract. Polycythemia was induced by a whole milk diet supplemented by cobalt, iron, copper and manganese.

The results on hand indicate that the administration of liver causes a brisk though not sustained fall in the erythrocyte count, which, however, reached the normal level in only a few cases.

On the measurement of skin temperature and body radiation. JAMES D. HARDY (by invitation). The Russell Sage Institute of Pathology in affiliation with the New York Hospital and the Department of Medicine, Cornell University Medical College. (Paper and demonstration.) (Read by title.)

A radiometric device for measuring skin temperature and body radiation has been developed which is portable, rugged, and accurate. The instrument embodies the "thermo-level" principle of Aldrich and has the advantage of being much simpler and easier to use. Radiation values are accurate to ± 0.1 small cal/sec/m², and skin temperature to $\pm 0.1^\circ\text{C}$.

Many of the ordinary instruments which are used in skin temperature and radiation work have been studied from experimental and theoretical points of view. The many errors incurred in such work have been traced as being inherent in the methods.

The radiating characteristics of the human body have been investigated and measurements have been made under various atmospheric conditions on several normal subjects.

Contraction types in the excised rat uterus and their relation to intra-uterine tension. O. G. HARNE (by invitation) and E. E. PAINTER (by invitation). Department of Physiology, School of Medicine, University of Maryland.

Contraction types in the excised rat uterus may be divided into two groups: 1, regular, including maximal and submaximal responses; 2, irregular, including all the composite forms.

The first group is observed in all excised uteri which before excision were distended, and in tonic empty uteri when the circular muscle is inactive, or if active, relaxes during the course of a longitudinal contraction.

The second group may be considered contraction types arising from disorganization of the two muscle systems, e.g., where there is no compensatory relaxation of the circular muscle during longitudinal shortening. This permits a rise in intra-uterine tension with each longitudinal contraction, which may reach 15 mm. Hg and involve either the entire horn or a local area, as between contracting rings of the circular muscles. Intra-uterine tension thus generated excites the uterus to activity, raises the muscle tonus and increases the contraction rate.

A peristaltic wave is capable also of interrupting the longitudinal con-

traction cycle, and thus produces steps at points in the contraction cycle where they pass. If the interruption lasts a sufficient time, and intra-uterine tension is maintained high (15 mm. or more), a new contraction or series of contractions occurs from a higher tonus level, and at an increased rate. These distortions of the contraction cycle coincide with either the passage of a peristaltic wave or the establishment of a high general or local intra-uterine tension.

Reflexes evoked by stimulation of fibres of lowest threshold. A. S. HARRIS (by invitation). Physiological Laboratory, Washington University School of Medicine.

The properties of various cutaneous nerves of the hind limb of the bull-frog have been studied by 1, stimulation and registration of the reflex response; 2, observations by means of the cathode ray oscillograph, and 3, histological methods. The nerve which has been most thoroughly studied is the ramus cutaneus cruris posterior of the tibial nerve, supplying the skin posterior to the gastrocnemius muscle.

The outstanding reflex response to repeated electrical stimulation of this nerve is ipsilateral extension. The threshold of this extensor reflex evoked by rapid stimulation is of the same order as the threshold of a motor nerve of the same frog. The conduction rate of the fastest fiber in the nerve has been found to be approximately the same as that of the fastest conducting fiber in the sciatic. The largest fiber is always about as large as the largest fiber in the sciatic, fibers of twenty-four microns diameter having been found in two of the many slides examined. Usually the largest fibers in both nerves are eighteen to twenty microns in diameter.

The findings on this nerve thus differ from earlier data with respect to the size and conduction rate of the largest fibers in cutaneous nerves. Both Erlanger and Sherrington found the largest fibers in skin nerves studied by them to be smaller than the largest fibers in muscle nerves. Correspondingly, Erlanger showed that the cutaneous nerves studied had slower conduction rates than did the fibers contributing to the alpha potential wave.

Some evidence has been collected on other cutaneous nerves of the leg and foot which indicates that they correspond with ramus cutaneus cruris posterior as to quality of reflex response and its threshold, size of the largest fiber, and conduction rate. These cutaneous nerves containing large rapidly conducting fibers mediate ipsilateral extensor reflexes of locomotion or posture.

The spectral sensitivity of single visual sense cells. H. K. HARTLINE and C. H. GRAHAM. Johnson Foundation, University of Pennsylvania.

The effect of various wave-lengths of visible light in the stimulation of single visual sense cells has been studied by means of the single fibre preparation from the eye and optic nerve of *Limulus*. Oscillographic records were made of the discharge of impulses in a single optic nerve fibre, in response to stimulation of the attached sense cell by light from different regions of the visible spectrum. Wratten monochromatic filters supplied the means for obtaining the spectral lights; the total intensity transmitted by each filter being determined by a thermopile and galvanometer.

The response of the single visual sense cell does not vary qualitatively with wave-length of stimulating light; by properly adjusting the intensity,

responses can be obtained which are identical, impulse for impulse, for all the spectral lights used. The reciprocals of these intensities necessary to produce a constant response, plotted against wave-length, give the visibility curve of the single sense cell. This curve is symmetrical about a maximum at *ca.* 520 $m\mu$, falling off to low values in the red and in the violet. It closely resembles the visibility curve of human rod vision, with the maximum somewhat displaced.

Bundles from the optic nerve, containing several active fibres whose impulses can be distinguished by differences in size and shape, were used to determine whether there is any differential sensitivity among sense cells of the same eye, in different regions of the spectrum. Such a differential sensitivity has been found to exist in the eye of *Limulus*, and may be considered a peripheral mechanism of color vision.

Water shift in adrenal insufficiency. F. A. HARTMAN, C. A. WINTER (by invitation), and K. A. BROWNELL (by invitation). University of Buffalo.

Muscles from adrenalectomized rats when placed in hypotonic salt solution imbibe water faster than those from normal animals. Similar muscles lose their water more rapidly in hypertonic solution than normal muscle. The more rapid passage of water in either direction through the cell membrane may indicate a greater permeability.

We have earlier demonstrated that adrenalectomy causes an increase in water content of skin in rats, and it has been shown by other workers that excretion of sodium chloride is markedly increased in this condition. Since chlorides play an important part in the osmotic balance of the body fluids, and therefore in the distribution of water to the various tissues, it was deemed important to study chloride shift in various tissues and thus determine which are most involved in the loss of total body chloride. In rats, skin, muscle, whole blood, and brain were studied. The only significant change was in skin, which showed a decrease of chloride content after adrenalectomy. A study of skin, muscle, plasma, and corpuscle chlorides in cats showed the most marked decrease in muscle after adrenalectomy, with lesser but perhaps significant decreases in plasma and corpuscles. There seems to be indication, in these findings, of a species difference in chloride shift after adrenalectomy.

Gastric acidity as influenced by exercise, psychic disturbance and anoxemia.

FRANCES A. HELLEBRANDT, ELIZABETH BROGDON (by invitation) and SARA L. HOOPES (by invitation). Department of Physiology, University of Wisconsin. (Read by title.)

Previous experiments had shown that strenuous exercise may at first depress the initial secretory response of the stomach to various test meals. The disappearance of the inhibitory effect as a result of the repetition of measured work has been confirmed. After a variable training period severe exercise may be carried on without disturbing the digestive work of the stomach. Experiments have been directed at an explanation of the initiatory restraining effect.

¹ Healthy young adults acted as subjects. By fractional analysis and continuous intubation the normal resting gastric secretory response was determined to 400 cc. of oatmeal gruel taken by mouth, to 50 cc. of 7 per cent alcohol administered via stomach tube, and to ergamine acid phosphate given subcutaneously in doses of 0.1 mgm./10 kilos body weight.

The effect of three conditions upon these responses was then studied: psychic disturbance, exercise and anoxemia. Emotional stress was relatively ineffective. Both exercise and anoxemia reduced the acid secretion which followed the ingestion of oatmeal gruel and of alcohol, but failed to significantly alter the response to histamine. The evidence suggests that the inhibiting effect of exercise upon the secretory power of the stomach is the resultant of a failure in the release of the gastric hormone rather than an effect upon the secreting cells themselves. If the stimulant to the secreting cells is artificially introduced, they function normally even in the face of marked anoxemia.

An amplified ballistic method for the measurement of the e.m.f. of the glass electrode. ALLAN HEMINGWAY. Department of Physiology, University of Minnesota.

For biological pH measurements the glass electrode has many advantages over the standard methods. The wide use of the glass electrode has been prevented by the difficulties involved due to the high electrical resistance of the membrane and the change of the membrane e.m.f. when a small amount of continuous current is drawn from the system. These difficulties have been eliminated by using the amplified ballistic method. In addition, the apparatus has the following advantages: 1, stability; 2, low cost of operation and of apparatus; 3, elimination of shielding; 4, rapidity of measurement; 5, accuracy with portable set to 0.02 pH.

The glass electrode assembly is connected in series with a potentiometer and to the plates of a condenser. A tapping key disconnects the glass electrode and connects the condenser across the grid resistance of the first tube of a two stage amplifier. A microammeter (full scale, 100 microamperes) in the plate circuit of the second tube records changes of plate current as a ballistic throw when the condenser is discharged. When the potentiometer voltage is equal to the voltage of the glass electrode assembly the ballistic throw is zero.

The vacuum tube filaments are supplied by current from four dry cells and the B batteries are of the smallest size. The two tubes are: a no. 34 in the first stage and a no. 49 in the second.

A potentiometer for the measurement of the e. m. f. of the hydrogen, quinhydrone and glass electrodes. ALLAN HEMINGWAY. Department of Physiology, University of Minnesota. (Demonstration.)

A portable potentiometer and vacuum tube circuit for the measurement of the e.m.f. of electrodes used for pH determination has been constructed using vacuum tubes operated on dry cells and using a low sensitivity galvanometer. The circuit is stable, no shielding of the glass electrode is necessary, and an accuracy of 0.02 pH is possible.

Asphyxial hyperpnea and the sinus caroticus. YANDELL HENDERSON and LEON A. GREENBERG. Laboratory of Applied Physiology, Yale University.

The evidence at present is that deprivation of oxygen acts upon respiration through the sinus caroticus. In accord with this view we find that after excision of both sinuses inhalation of pure nitrogen induces no increase of breathing during the first 40 to 60 seconds of the asphyxia. But we find also that, if the asphyxia is continued into a second minute, hyperpnea does develop.

The effect of the digestion of food on the blood flow from the liver of the dog.

JULIA F. HERRICK, F. C. MANN, HIRAM E. ESSEX and E. J. BALDES.

The Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Modification of the thermo-stromuhr method of Rein has made possible observations on the blood flow from the liver over periods of one to eighteen days following placing of the unit (diathermy-thermo-element) on the blood vessel. The surgical procedures necessary to permit the blood flow from the liver to be measured in a single vessel (thoracic portion of the posterior vena cava) have been described in a previous abstract. Briefly it consists of occluding the posterior vena cava just caudal to the entrances of the hepatic veins. The blood flow from the liver has been observed following fasting and during the digestion of food. In each experiment observations were made for at least one hour before feeding. A meal composed of milk (250 cc.), eggs (2) and glucose (1 gram per kgm.) produced an average increase in blood flow of about 54 per cent. The highest flow occurred during the second or third hour following the meal and a return to about the initial level required about five hours. In a dog weighing 7.8 kgm. the blood flow from the liver on three successive days was 500 cc., 552 cc. and 408 cc. per minute. The maximum increases following a milk-egg-glucose meal were 890 cc., 885 cc. and 655 cc. respectively. A meal of cooked meat and cereal produced as much as a 74 per cent increase in blood flow with a maximum during the second hour after feeding and a return to about control value in six hours.

Reactions of blood-vessels of the brain-stem and spinal cord and their relation to circulatory and respiratory regulation.

ALRICK B. HERTZMAN and FLORENT E. FRANKE (by invitation) with the assistance of L. D. SEAGER (by invitation) and E. SOMKIN (by invitation). Department of Physiology, St. Louis University School of Medicine, St. Louis.

Blood-vessels on the surface of the pons, medulla and cervical portion of the spinal cord of the dog under morphine-urethane anesthesia have been observed through specially designed windows inserted through the floor of the cranium and atlanto-occipital membrane retaining the closed-box mechanics of the cranium and vertebral column. Photography using shutter times from 0.01–0.002 second, permitted simultaneous measurement of arteries and veins with an error of 0.005 mm. or less. Vessels varying from 0.015 mm. to 0.350 mm. have been studied. The majority of vessels have been in the size range 0.06 mm. to 0.16 mm.

The following statements are based on 5000 measurements on 47 arteries and 29 veins in 14 dogs during 64 experimental disturbances in the circulation, respiration and blood chemistry. 1. These blood-vessels have been remarkably unresponsive, their diameters often remaining constant within the limits of error of measurement. Changes in diameters have been usually less than 0.1 mm. although in a few cases they have been larger. 2. Small arterial anastomoses were more reactive, changes in diameter as large as 30 per cent being observed in several cases, the anastomosis sometimes showing local constrictions or dilatations. 3. When changes in diameter occurred, they appeared to be passive, running parallel to the changes in arterial pressure. There were very few exceptions to this. These observations stand in sharp contrast to the reactivity of the pial vessels of the cerebrum.

Our data are in accord with the concept that the blood supply of the

brain-stem is primarily dependent on arterial pressure and is not subject to an important local control, nervous or chemical. We do not extend this concept to other parts of the brain.

We have seen large changes in the blood-flow through the vessels of the pons and medulla in several instances (e.g., occlusion of vertebral arteries) to be without noticeable effect on the heart rate, blood pressure and respiration. This suggests that the activities of the circulatory and respiratory centers may be relatively undisturbed by changes in their blood supply within wide limits.

Ketogenic diets. The means of eliminating acids by the human kidneys.

HAROLD L. HIGGINS and ANGELIA COURTNEY (by invitation). Harvard University.

Data are presented of twelve observations, each extending over several weeks, in which a high fat (ketogenic) diet was fed to children. In the early stages of the diet the hydrogen ion concentration, the titratable acidity and the phosphate of the urine are increased; later these constituents become smaller, but the urinary calcium, ammonia and total organic acids increase.

Observations on skin temperatures in hind limbs deprived of their sensory and sympathetic nerve supply. J. C. HINSEY. Department of Anatomy, Stanford University. (Read by title.)

The right hind limbs of five adult cats were deafferented by section of appropriate dorsal roots. Skin temperatures (thermocouple, and thermometer between the foot-pads) were followed for a year on corresponding areas of the shaved skin on the medial surface of the thigh and from the foot-pads. The right limb (particularly foot) was significantly cooler than the left (less evident in a warm room 23–28°C. than in a cool room 15–22°C.). This could be felt by disinterested observers. After a year, these animals were subjected to a right abdominal sympathectomy (L2–L7). Immediately following the sympathectomy, the right limb, which had been cooler, became warmer (as much as 6–7°C.) than the left normal one, and in most instances still remains so after 20 months, although differences are not so great (1–2°C.) in some.

In another series, 3 cats were sympathectomized (right abdominal). One year later, the right limb (in a cool room) was significantly warmer (2–4°C.). Then two of these cats were deafferented by section of appropriate right dorsal roots. The right foot still remained significantly warmer in both instances and still continues to be after 18 months (2–4°C., thermocouple on foot-pads in room 15–17°C.). A similar difference has been seen in a cat that was subjected to a unilateral abdominal sympathectomy 17 months ago.

Since our observations are continuing, no autopsies have been made. Deafferentation alone causes a reduction in skin temperature on the operated side. This seems to be due to an overactivity of the sympathetic vasoconstrictors because it disappears and gives way to a warmer than normal limb following sympathectomy. Vessels that have been sympathectomized for as long as 30 months have not regained their tonus to a degree enabling them to cope in a normal manner with conditions in a cool room. The importance of room temperatures, emphasized by Reichert (1932, *Proc. Soc. Exp. Biol. and Med.*, **29**, 473), is confirmed. The

differences are more marked in the foot-pads than in the more proximal portions of the limb.

The physiological control of judgments of duration. HUDSON HOAGLAND. Physiological Laboratory, Clark University.

Judgments of short durations, as determined by counting seconds, vary with the internal body temperature and appear to be described by the Arrhenius equation over the range of 3.2°C. The speed of counting increases with temperature. A temperature characteristic of 24,000 calories is found for my own data and for those of François. These sets of data were taken independently, for different purposes, and by the use of different experimental procedures. It is suggested that judgments of duration may depend on the velocity of a particular chemical reaction (clock) in the nervous system, probably irreversible in nature, and catalyzed in a particular manner corresponding to $\mu = 24,000$ calories. Sensorimotor activity apparently increases the absolute speed of the chemical clock without changing the value of μ .

The effect of temperature on the time sense is found to be very similar to its effect on certain processes of central nervous origin in cold-blooded animals. Motor activity has been found to modify these processes in a way similar to that in which it modifies the time sense. The value of $\mu = 24,000$ calories is common to a variety of biological systems and is generally associated with respiratory phenomena in cells.

Physiological factors involved in the estimation of long durations are discussed.

Corticospinal fibres originating in the premotor area and their terminal distribution. E. C. HOFF. Laboratory of Physiology, Yale University.

The *boutons terminaux* occurring on neurones of the central nervous system undergo degenerative hypertrophy and granular disintegration following section of their axones (E. C. Hoff, Proc. Roy. Soc., 1932, B exi, 175). This synaptic degeneration, which reaches a maximum three days after the lesions, has now been used to investigate the terminal distribution of fibres originating in the premotor area (Brodmann's area 6).

In normal adult monkeys (*Macaca mulatta*), area 6 was removed aseptically. Forced grasping and other manifestations of premotor injury were present, and subsequent histological examination demonstrated that there had been no damage to Betz cells. Study of the cords of these animals, sacrificed 72 hours after operation, revealed degenerating *boutons* in the entire cord as far caudally as the lower lumbar segments. As shown by this degeneration, corticospinal fibres from area 6 terminate chiefly in the mid-region of the contralateral gray matter somewhat more mesially than do fibres from the motor area. Some fibres end around ventral-horn cells directly. Degenerating *boutons* are found more sparsely on the uncrossed side both in the mid-region and in the ventral horn.

Bouton degeneration was also studied in monkeys in which superficial cortical incisions were made between motor and premotor areas with histological confirmation that there had been no encroachment upon the motor area. In these experiments the degeneration was similar to that found upon complete removal of the premotor area. Many fibres from area 6, therefore, run into the motor area and proceed into the cord with the pyramidal fibres. On the basis of the richer *bouton* degeneration after complete

destruction of area 6, it is probable that extra-pyramidal corticospinal fibres from the premotor area also exist. This is in harmony with Bucy's observations (Arch. Neurol. Psychiat., 1933, 30, 1205) that the premotor area is excitable after the motor area is destroyed.

Further studies on amino acids in development. O. HOFFMAN (by invitation) and F. GUDERNATSCH. New York University, Washington Square College, New York City.

In previous studies on development we found, when feeding a N free basal diet plus α -amino acids, maintenance best in glycine, alanine or leucine (M acids); growth in arginine, lysine, cystine or cysteine (G); differentiation in phenylalanine, tyrosine or tryptophane (D). Further experiments aimed to determine which acid mixtures, if any, would best facilitate development, total acid concentration being the same (1:1000) in all mixtures. *Rana sylvatica* tadpoles (two sets) were used.

Set A—1. 1 G (cysteine) + 1 M (alanine; leucine) — good maintenance, quite good growth, in alanine some differentiation.

2. 1 G (cysteine) + 1 D (phenylalanine; tyrosine; tryptophane) — poor maintenance, fair growth (tyrosine > tryptophane > phenylalanine) some differentiation.

3. 1 G (cysteine) + 1 M (alanine; leucine) + 1 D (phenylalanine, tyrosine; tryptophane) — poor maintenance, quite good growth, good differentiation. Alanine + phenylalanine and leucine + tryptophane were the poorest groups.

4. 2 G (cysteine, arginine) — poor maintenance, quite good growth, poor differentiation.

5. 2 G (cysteine, arginine) + 1 M (alanine; leucine) — good maintenance with leucine, not with alanine; quite good growth, little differentiation.

6. 2 G (cysteine, arginine) + 1 D (tryptophane) — good early maintenance, very good growth, quite good differentiation.

7. 2 G (cysteine, arginine) + 1 M (alanine; leucine) + 1 D (phenylalanine; tyrosine; tryptophane) — poor maintenance, except alanine + tryptophane; growth in general good, not in alanine + phenylalanine, nor in alanine + tyrosine; differentiation moderate when life span long enough.

Set B—1. 2 G (cysteine; arginine; lysine; in combinations of two) + 2 D (phenylalanine, tryptophane) — maintenance, growth, differentiation best in cysteine + arginine + phenylalanine + tryptophane.

2. 2 G (see 1) + 2 D (phenylalanine, tyrosine) — maintenance, growth best in cysteine + lysine; differentiation in arginine + cysteine.

3. 2 G (see 1) + 2 D (tyrosine, tryptophane) — maintenance best in cysteine + lysine + tyrosine + tryptophane; growth poor in all; differentiation best in cysteine + arginine + tyrosine + tryptophane.

In entire set B differentiation was best in cysteine + arginine + tyrosine + tryptophane; next in cysteine + arginine + phenylalanine + tryptophane; next in cysteine + arginine + phenylalanine + tyrosine.

While these data allow no final conclusions, the weight of evidence points in the same direction as previous observations, viz., that specific amino acids are of specific value for either maintenance, growth or differentiation. Number and variety of acids in a mixture determine the physiological value of the mixture for the normal progress of development.

Direct determination of the freezing point of normal mammalian and neoplastic tissues. EVELYN HOWARD. Laboratory of Physiology, Johns Hopkins University School of Medicine.

Freezing point determinations on tissues have been made by means of thermocouples inserted between pieces of isolated tissue. Samples of tissue are removed from rats or rabbits under amytal anesthesia, and quickly inserted into precooled tubes at 1 to 2°C. to minimize autolytic changes in the tissue. Reduction of the animal's temperature to 26° (rectal) by means of ice packs under anesthesia was easily accomplished and was employed to reduce tissue metabolism and to facilitate the cooling of the isolated tissue. With these precautions it was found possible to obtain tissue freezing points which agreed with that of the blood to within 0.00 to 0.05°C., depending on the kind of tissue. If the animal is killed by a blow on the head, tissue freezing points are higher than when tissue is obtained under amytal anesthesia. If the tissue is allowed to stand for thirty minutes at room temperature, the tissue Δ may rise to nearly double that of the blood. Hence freezing point determinations should be of value as a check on the amount of autolysis occurring during tissue removal for analyses. Autolysis, as determined by the increment in freezing point depression, is considerably greater for liver than for brain, salivary gland, testis, or neoplastic tissue.

Various rat sarcomata and the Brown-Pierce rabbit carcinoma were found to have normally the same freezing point as blood in spite of the fact that they have a higher water content than adult somatic tissue. Hence the difference in water contents between normal and neoplastic tissues is not maintained as a result of a difference in the potential osmotic pressure of the total molecular constituents.

The blood sugar level after insulin and epinephrine. M. CAROLINE HRUBETZ (by invitation). The Department of Physiology, Columbia University, New York City.

With 1300 observations on the blood sugar level of rats 20 minutes, 40 minutes, 1, 1½, 2, 2½, 3, 3½, 4, 5 and 6 hours after the injection of ¼ unit of insulin per kilo, the greatest drop occurs in the first 20 minutes. The curve reaches its lowest point 1 hour after injection. In 6 hours the curve has not returned to the original level, due, probably, to the effects of inanition.

With 1000 observations made at 5, 15, 30, 45 minutes, 1, 1½, 2 and 4 hours after given doses of epinephrine, the blood sugar reaches its highest level in 1½ hours and has not returned to the normal level in 4 hours.

In another experiment, a series of 50 observations was made on normal-fed rats every 3 hours during the day. The means of the two series check within 2 mgm. The failure of the above curves to return to normal in the allotted time cannot be attributed to diurnal variation.

Urethral clonus. C. B. HUGGINS, H. R. MCCARROLL (by invitation), and H. E. HAYMOND (by invitation). Department of Surgery, University of Chicago. (Read by title.)

A striking rhythmical contraction of the membranous portion of the urethra in male dogs was observed when the urethra was distended with fluid. This clonus was best observed when the abdominal cavity was opened under light ether anesthesia, the urinary bladder compressed

manually, while the anterior urethra was simultaneously occluded with the fingers, so that urine was forced into the urethra distending it. At a tension of 15 to 30 mm. Hg, rhythmical movements occurred and continued for as long as 5 minutes: following the release of pressure there is an "after discharge" while the intra-urethral tension is falling. The rhythmical contractions were abolished by 1, moderate or deep ether anesthesia; 2, section of the medullated internal pudic nerves. The rate was fairly uniform, about 3 contractions per second. Compression of the bladder was not essential for development of the clonus: it occurred when the membranous urethra was distended by injecting fluid into it through a catheter in the urethra. The clonus was not observed in female dogs. Relationship to mechanism for ejaculation of semen.

Changes of electrical resistance in nerve during block by cold. I. F. HUMMON, JR. (by invitation) and T. E. BOYD. Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago.

Using alternating current from a vacuum tube oscillator, with a bridge and telephone, we have followed the changes of resistance in frog nerve subjected to local cooling. In nerves which are blocked by cold without freezing, the curve relating resistance to temperature shows no marked change in slope at the time when impulse conduction stops. With a freezing block, the resistance curve becomes suddenly steeper as ice formation begins. In supercooled nerves (-4.0°C . or lower) the rise of resistance at the moment of block is extremely rapid, and the final resistance high (confirming Bahrmann, *Zeitschr. f. Biol.*, 1932, 92, 336). Recovery on warming is delayed or absent. Nerves frozen at -1.0 or -1.5°C . show, by comparison with the supercooled group, *a*, a slower rise of resistance as block develops; *b*, a relatively low final resistance when block is complete; *c*, immediate recovery of conduction on warming.

Effects of lesions in the hypothalamus in cats. W. R. INGRAM, C. FISHER and R. W. BARRIS (by invitation). Northwestern University Medical School.

In a series of 57 adult cats bilateral, symmetrical lesions were placed in the hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument. With this instrument it is possible to place localized lesions in any part of the hypothalamus without injury to the adjacent stalk of the pituitary or pars tuberalis.

The effects of these lesions on urine excretion and water intake, appetite and adiposity, blood sugar, temperature regulation, urine and blood chlorides and general behavior are being studied in carefully controlled experiments.

In general these animals show greatly subdued behavior following operation, but in many cases perfectly normal behavior is evident in spite of extensive damage. Rather extensive lesions do not cause death. Blood sugar determinations indicate a transient rise following operation, with subsequent return to normal levels. Similarly, transient rises in temperature are frequent. In a few cases transient hyperthermias have been observed.

Thus far it cannot be said that lesions causing extensive damage to the inferior-medial part of the hypothalamus between the chiasma and the mammillary bodies produce a derangement of fat metabolism which leads to adiposity.

Permanent diabetes insipidus has been produced in four animals and a mild permanent polyuria in two others. The urinary output in these animals is balanced by the fluid intake and exceeds the normal values by as much as five or six times. The polyuria and polydipsia can be suppressed by repeated, small injections of Pitressin or by depriving the animals of water. Starvation with free water supply reduces the urine output by about half.

The brains of two of these cats, sacrificed after two months, show restricted lesions in the inferior-medial part of the hypothalamus between the optic chiasma and mammillary bodies, involving the tractus hypothalamico-hypophyseus. In both cats the supra-optic nuclei were atrophied. However, we are not prepared to say that this nucleus is the center for the regulation of water metabolism.

Control of the circulation through the brain according to the composition of inspired air. LAURENCE IRVING and MARY SCOTT WELCH (by invitation). Department of Physiology, University of Toronto.

Central nervous tissue is particularly sensitive toward asphyxia. In compensation, it has been shown (Lennox and Gibbs, Journ. Clin. Invest., 1932, 11, 1155) that the human cerebral circulation is preferentially accelerated by carbon dioxide inhalation. The proposition was examined in dogs under dial anesthesia in order to look for details of this significant type of reflex vascular control. Blood samples were taken simultaneously from the brachial artery, femoral vein and longitudinal sinus. Examination of CO_2 and O_2 content permitted calculation of the normal (anesthetized) A. V. difference. Then, following inspiration of various mixtures of N_2 , O_2 and CO_2 , a second series of samples showed the new A. V. difference. The change was caused by the alteration in rate of blood flow through the hind leg and brain respectively. The results indicated that inspiration of CO_2 rich mixtures increased the cranial but not the femoral flow. Cranial flow is not so sensitive to O_2 lack. The paths of stimulation involved in this reflex differential control of the circulation are examined. The bearing of the results upon survival during apnea is also considered.

The influence of electrolytes on the rate of hemolysis in glycerol solutions. M. H. JACOBS, A. K. PARPART (by invitation) and S. A. CORSON (by invitation). University of Pennsylvania.

Previous experiments by the hemolysis method indicate that electrolytes in very low concentrations retard the entrance of water into the erythrocyte to an extent that cannot be accounted for by the osmotic pressure of the external solution alone. The present investigation deals in a similar manner with the more complicated case where penetration of the erythrocyte by a solute as well as by water is involved. It is found that in solutions of glycerol so dilute that hemolysis can occur rapidly and with little or no entrance of the solute, the effect of electrolytes is the same as that previously reported; with more concentrated solutions, where hemolysis is possible only after a considerable degree of penetration by the solute, electrolytes in low concentrations accelerate instead of retarding the process; in sufficiently high concentrations they invariably delay it through a direct osmotic action. By various combinations of these three effects a great diversity of results is possible with only slight differences in experimental conditions. Evidence is given which indicates that the apparent

effect of electrolytes on the penetration of the erythrocyte by a non-electrolyte is not primarily one of cell permeability, as commonly understood, but rather an indirect one of osmotic nature. The results reported support the view advanced elsewhere that in erythrocytes exposed to a non-electrolyte solution an exchange of Cl^- and HCO_3^- ions from the cell for OH^- ions from the solution lead to an increase in the base bound by hemoglobin and in consequence to a reduction of the internal osmotic pressure and to a shrinkage of the cell. It is apparently through osmotic volume changes of the cell, resulting from altered ionic equilibria of this sort, that some of the most striking of the observed electrolyte effects on the rate of hemolysis are produced.

The effects of extirpation of the frontal association areas in monkeys upon complex adaptive behavior. C. F. JACOBSEN (by invitation). Laboratory of Physiology, Yale University.

Monkeys were trained in two types of behavioral tests: 1, situations in which the essential cues to correct response were present in the animal's immediate sensory experience and could be referred to by the animal while responding, i.e., simple problem boxes, and discrimination of brightness and size of visual patterns; and 2, situations in which the essential cues, wholly or in part, had to be recalled from the animal's past experience, i.e., delayed response (the animal saw food placed under one of two cups but was prevented from seeking food for several seconds or minutes), and a serial problem box which required manipulation in a definite order.

Unilateral lesions of the frontal association areas resulted in no impairment of performance in any test. Complete bilateral extirpations (of the frontal areas) did not affect simple problem boxes or visual discrimination habits, but did result in stereotypy of performance on the serial problem box and complete loss of the ability to delay a response for even a few seconds. Incomplete bilateral lesions resulted in serious reduction in ability to react successfully after long delays although the animal continued to respond correctly if the delays did not exceed a few seconds. Bilateral lesions in the frontal association areas greatly interfered with behavior dependent upon recalling recent experience (immediate memory), but did not impair behavioral adaptations determined by cues present in immediate sensory experience.

With bilateral lesions in the parietal association areas, of approximately equal extent, there was no reduction of the ability to perform the delayed response tests. Similar experiments by Breslau on the postcentral gyrus gave uniformly negative results. It is tentatively suggested that the ability to respond on the basis of "immediate memory" is peculiarly dependent upon the intactness of the frontal association areas. The significance of these experiments for theories of localization of function in the cortex will be considered.

*Further observations of the monophasic action currents.*¹ KENNETH JOCHIM (by invitation), LOUIS N. KATZ and WALTER MAYNE (by invitation). The Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Chicago. (Read by title.)

¹ Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

Monophasic action currents were obtained in direct leads from the mammalian ventricles by using one of the electrodes to create the injury. This electrode consisted of a glass capillary tube (a millimeter in bore) containing a wool wick moistened with saline. When pressed lightly against the surface of the heart it produced sufficient injury to give monophasic curves which persisted as long as the pressure was maintained.

It was found that the duration of the monophasic curves obtained by connecting one injured area with various uninjured regions of the heart varied as much as 0.06 of a second with the heart rate constant, a difference adequate to explain the genesis of the T wave as postulated by Katz. K or Ca when applied to the uninjured spot altered the duration of the monophasic curve, but had no effect when applied to the injured area.

The variation in duration obtained by connecting different uninjured regions with the injured region is difficult to reconcile with the concept recently put forth by Wilson that the monophasic action current measures the potential changes in the injured area and not those in the uninjured area. Our results support the more generally accepted point of view that the monophasic curve is an expression of the "depolarization-repolarization" changes occurring in the uninjured regions.

The effect of viosterol on the calcium content of the bile. KENNETH K. JONES and GRANT H. LAING (by invitation). Department of Physiology and Pharmacology, Northwestern Medical School.

Since calcium is one of the constituents of gall stones, it is important to know the effect of viosterol on the amount of calcium in the bile.

In order to study the effect of viosterol, varying amounts of standard preparations were given to ten dogs with a bile fistula. This fistula was made by cannulating either the common bile duct or the cystic duct with the removal of the gall bladder. This made possible the collection of the total hepatic bile daily.

Viosterol was given by mouth, either with or without the addition of bile salts, and also subcutaneously and intravenously.

Calcium in the bile was estimated by ashing the bile, dissolving the ash in hydrochloric acid, precipitating with ammonium oxalate in neutral solution, filtering off the calcium oxalate and titrating with potassium permanganate in dilute sulphuric acid.

The calcium in the bile is high (18-20 mgm. per 100 cc.) following the operation in most dogs but falls to a basal level in two to three days. When the dog is normal and in good state of nutrition with sufficient calcium in the food, the bile calcium becomes steady at 14 mgm. per 100 cc.

This is not affected by large therapeutic doses of viosterol (50 drops per day of 250 D viosterol given by mouth with bile salts).

The calcium does not increase in the bile until enough viosterol is given (1 cc. 10,000 x viosterol) intravenously to raise the blood serum calcium above normal. Then there is a rise in bile calcium to 60 mgm. per 100 cc. when the blood serum calcium reaches 20 mgm. per 100 cc. which is accompanied by a marked decrease in the total bile excreted.

The number of reticulocytes in the blood of emotionally excited rabbits. H. L. KATZ (by invitation) and L. B. NICE. The Ohio State University.

On a series of blood smears made from the ear veins of rabbits in the quiet and excited state, the per cent of reticulocytes was determined.

All smears were stained with a saturated saline solution of Bromcresol Blue and counterstained with Wright's stain. The per cent of reticulocytes was determined by counting the number of these cells in 1000 erythrocytes.

On specimens of these bloods, hemoglobin determinations and total erythrocyte counts were made.

In 17 experiments on normal rabbits 13, or 77 per cent, showed an increase in the total reticulocytes, as a result of excitement, but no increase in the per cent of these cells in the blood stream; while in four cases negative results were obtained. There was an increase in hemoglobin in 81 per cent.

In six experiments on splenectomized rabbits, 83 per cent showed an increase in the number of reticulocytes after excitement with an elevation in the per cent. There was no change in hemoglobin and total erythrocytes.

These results are interpreted as indicative of increased bone marrow activity in the rabbit during excitement with the consequent inpouring of reticulocytes into the blood stream.

*The importance of the extravascular force exerted by the cardiac muscle on the flow of blood in the coronary vessels.*¹ LOUIS N. KATZ, KENNETH JOCHIM (by invitation) and ANNE BOHNING (by invitation). The Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Anrep's contention that the only important factor influencing coronary flow is the diastolic aortic pressure has been questioned recently by Hochrein, Rein and Wiggers. The evidence, pro and con, is still indirect. It seemed to us that the problem might be settled by determining coronary inflow and coronary sinus outflow simultaneously under different dynamic conditions.

A modified heart-lung preparation was used. The three main branches of the coronary arteries were cannulated and perfused with blood under constant pressure. The coronary sinus was drained with a Morawitz cannula. In this way the coronary system was separated from the cardiac chambers except for accessory coronary veins and Thebesian communications. The dynamic conditions of the heart were altered by varying the venous and arterial pressures. The heart rate was kept constant by electrical stimulation.

The following conclusions are suggested by the preliminary experiments:

1. The flow into the coronary arteries decreases if the systolic and diastolic pressures of the heart chambers are increased by raising the venous pressure and the arterial resistance, the coronary pressure and heart rate being kept constant. This indicates that the compression exerted by the heart muscle on the intramural vessels can modify the coronary blood flow. The effect of this compression apparently depends on the tension-time curve of the various heart chambers.

2. Under these conditions the coronary sinus outflow, contrary to Anrep's conclusion, does not bear a constant relationship to the coronary inflow. The coronary sinus is only one of the pathways by which blood is

¹ Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

drained, and its proportion of the total drainage varies as dynamic conditions are altered.

3. The coronary outflow under certain circumstances may exceed the coronary inflow, confirming the results previously reported by us that the Thebesian communications can serve as a portal of entry for blood to the myocardium as well as a portal of exit.

*The manner in which the electric currents generated by the heart are conducted away.*¹ LOUIS N. KATZ and HERMAN KOREY (by invitation). The Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Chicago. (Read by title.)

During the course of experiments in which volume curves were recorded simultaneously with standard lead electrocardiograms, it was found that the use of a glass-rubber oncometer to enclose the ventricles decreased the amplitude of the ventricular complexes to such a degree that, in some instances, they were barely visible. A similar result was obtained by completely ensheathing the heart with rubber sheeting while maintaining the heart's position unaltered. Further investigation showed that insulating the anterior chest wall from the heart had little influence on the electrocardiogram, but insulating the posterior wall of the chest from the heart had a marked effect. Insulating the diaphragm from the heart had an intermediate effect. Insulating the lungs from the heart had no appreciable effect on the electrocardiogram. Similarly, ligating and cutting the large vessels from the heart, viz., the venae cavae, the aorta and the azygos vein, had little influence on the electrocardiogram.

These results indicate that: 1, the large blood vessels (aside from the pulmonary vessels) and the blood contained within them are not of great importance in conducting the electric currents generated by the heart. 2. The lungs are also relatively unimportant in this regard. 3. The best conductors for the electric currents created by the heart are, in ascending order of importance, the anterior chest wall, the diaphragm and its adjacent viscera, and, most important of all, the muscle mass of the posterior chest wall in contact with the heart. This being the case, it is difficult to comprehend how vector analysis as promulgated by Einthoven and his followers can approach even an approximate picture of the electrical field as it actually exists. Alterations in the electrocardiogram may in some instances be produced not by changes in the "manifest potential" but rather by changes in the position of the heart with relation to the various conductors in contact with it.

The distribution and recovery of intravenously injected sucrose. NORMAN M. KEITH, M. H. POWER and RUTH D. PETERSON (by invitation). Division of Medicine and the Section on Clinical Metabolism, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

The value for the sucrose content of the blood immediately after injection, and for the sucrose appearing in the urine during the injection period, accounts for less than one-half of the amount injected. Sucrose therefore disappears rather rapidly from the blood stream, probably into the tissue fluids. Analysis of various tissues of the dog shows this to be

¹ Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

the case. On the other hand, sucrose appears to be unable to penetrate the red blood cells in man to any appreciable extent.

After the injection of sucrose in men, the recovery in the urine is practically quantitative, although delayed in certain types of renal disease. In the dog, however, we have recovered in the urine only 70 to 80 per cent of the quantity injected. The recovery in normal men during a definite period after injection is quite constant, and parallels the simultaneously determined value for the plasma sucrose clearance. It appears possible that the determination of the urine sucrose for a definite one-hour or two-hour collection period may be of considerable use as a somewhat simpler test of kidney function than the determination of clearances.

Ulceration in the digestive tract following hypophysectomy. A. D. KELLER, M. C. D'AMOUR (by invitation) and W. K. HARE (by invitation). University of Alabama.

Ulceration of the gut, essentially the same as that observed after hypothalamic lesions (Proc. Soc. Exp. Biol. and Med., 1933, **30**, 772) has been encountered in 8 of a series of 18 hypophysectomized dogs.

In 4 instances, the body of the stomach was markedly hyperemic, while the fundus and pylorus were relatively free from reaction. The mucosa of the small bowel was distinctly hemorrhagic in regions, especially in the duodenum. After operation, formed feces were passed followed by a bloody mucous diarrhea. The dogs died after approximately 24 hours.

In the other 4 cases, craters were found in stomach and duodenum. These were unassociated with hyperemia or hemorrhage. The gastric craters were few, small and shallow, and occurred in the pylorus as well as in the body. The presence of large deep twin ulcers in the proximal duodenum, the one rounded with regular edges, the other irregular in shape, was the most striking feature in each case. The animals died in typical cachexia hypophysopriva 4, 9, 16, and 17 days after operation.

It should be mentioned that all the dogs of the series that survived more than 24 hours developed this cachexia either spontaneously or after a short period of starvation. It was found that close attention to feeding was necessary to prevent its development. In most cases recovery was obtained by feeding or by injection of glucose subcutaneously. The time of onset of the prostration ranged from a few days to 8 months after operation.

Serial section of the hypothalamus of the 8 dogs described above failed to demonstrate any damage to the brain stem.

A series of experiments is now in progress to determine the mechanism by which the pathological changes are produced. Hypophysectomy is performed after the following procedures: 1, bilateral vagotomy in the thorax; 2, bilateral removal of the lower thoracic portion of the sympathetic chain (splanchnics), and 3, bilateral vagotomy plus bilateral removal of the splanchnics. Thus far, one of each of the following cases has been observed: 1, marked hemorrhagic involvement of the gastric mucosa after vagotomy; 2, shallow gastric craters after removal of the splanchnics; 3, cachexia hypophysopriva after vagotomy plus removal of the splanchnics.

Nussbaum's experiment. RUDOLF T. KEMPTON (by invitation). Laboratory of Pharmacology, University of Pennsylvania.

In Nussbaum's experiment, complete ligation of the renal arteries in the

frog did not prevent the elimination of urine after injections of urea. In exact repetitions of this experiment with two species of American frogs the same result was obtained. Examination of the kidneys during life showed, however, that when this result was obtained visible glomeruli contained circulating blood. This is due, not to incomplete ligation, but to the fact that when the posterior renal artery is tied, blood flow in it and in the glomeruli supplied by it is not prevented.

The collateral channels responsible for this have been identified. Urea is effective in augmenting blood flow through these channels. Injection of the aorta at the conclusion of the experiment is an inadequate means of proving the presence or absence of circulation in glomeruli.

The effects of thyroid administration upon motor conditioned reflexes in dogs.

N. KLEITMAN and S. TITELBAUM (by invitation). Department of Physiology, University of Chicago.

Daily oral administration of 2.0 gram doses of desiccated thyroid for fortnightly periods, alternating with similar control periods, to 10 kilo dogs, showed a definite effect of the glandular extract upon the unconditioned, positive conditioned, and negative conditioned motor reflexes. Three experimenters worked independently, each with one dog. The unconditioned reflex used was the withdrawal response to an electrical shock applied to the left hind leg. The dogs were conditioned to raise their legs when a metronome was sounded at a frequency of 100 beats per minute (positive conditioned response). By not reinforcing a frequency of 200 metronome beats, this frequency was established as a stimulus to be differentiated from the 100 beats and not responded to (negative conditioned response); this was alternated with the positive conditioned stimulus in an irregular order. As the dogs learned to differentiate perfectly between the positive and negative conditioned stimuli, the frequency of the latter was decreased so as to approach that of the positive stimulus. This procedure was repeated until for each dog the differentiation range was too small to permit of 100 per cent correct discrimination. At this stage the administration of thyroid was begun, and each dog went through two thyroid-feeding periods.

Generally, the percentage of correct responses to the positive conditioned stimulus and to the negative conditioned stimulus was increased during periods of thyroid feeding, and somewhat decreased during control periods. Thus, in one dog, to a negative conditioned stimulus of 130 beats, the percentages of correct responses during the control periods were 80.0, 87.3, and 89.0, while during the interspersed thyroid periods they rose to 89.7 and 92.7. The height of the responses to the unconditioned stimulus and to the positive conditioned stimulus was affected in a similar manner.

Colorimetric detection of barbituric acid derivatives. THEODORE KOPPANYI, WILLIAM S. MURPHY (by invitation), JAMES M. DILLE (by invitation) and STEPHEN KROP (by invitation). Georgetown University, School of Medicine, Washington, D. C. (Demonstration.)

Three methods of colorimetric estimation of barbiturates were developed in this laboratory.

A. Barium hydroxide, or "macro" method. This test consists of adding graded amounts of a one per cent solution of cobaltous acetate in absolute

methanol, and of a one per cent solution of barium hydrate in absolute methanol to chloroform extracts, and comparing their color with that of standards.

B. Lithium hydroxide, or "micro" method. Lithium hydrate is substituted for barium hydrate, and the test performed with more dilute reagents (0.2 per cent). The test is ten times as sensitive as the barium hydrate test.

C. Isopropylamine method. In this test a 5 per cent solution of isopropylamine in absolute methanol is substituted for the metallic hydroxide. The resulting colors are more stable than those obtained in the two previous tests, and are thus better adapted for standard colorimetric use.

Experimental analysis of barbital action. THEODORE KOPPANYI and W. S. MURPHY (by invitation). Georgetown University, School of Medicine, Washington, D. C.

In previous researches we have established the rate of disappearance of barbital from the blood, and its rate and percentage of elimination in the urine in various animals. We recently discovered a very effective method (intravenous medication with ammonium chloride) to accelerate the elimination of barbital from the body to such an extent that within a few hours half of the injected dose may be recovered from the urine.

It was found that at the time when more than half of the minimum anesthetic dose was eliminated the animals (dogs) failed to awaken. This result necessitated a new series of experiments on cats and rabbits. Thus far four cats and five rabbits have been used. The experiments consisted of intravenous administration of 150 mgm. of sodium barbital per kgm. at 24 hour intervals. Blood and complete urine samples were collected before injections.

The depth of depression of cats increased each day although the cats had eliminated increasingly larger percentages of the dose still remaining in the body. Accordingly the blood barbital became gradually lowered. On or about the fourth day of administration cats had eliminated over seventy per cent of the total dose given, retaining a dose of appreciably less than 100 mgm. of sodium barbital in the body. Such cats at this stage were unable to stand, walk or eat, and showed depression, whereas the dose remaining in the body would be insufficient to cause any appreciable effect if injected intravenously into other cats.

Rabbits treated in a similar manner showed a progressively decreasing elimination; thus the dose in the body as expressed in milligrams per kilogram, increased correspondingly. On or about the fifth day the dose remaining in the body exceeded the levels of the average fatal dose, and yet the animals were able to stand, walk, and eat. The blood barbital showed progressive rise.

Cats, therefore, up to a certain extent showed cumulation of effects without cumulation of dose, while rabbits showed cumulation of dose without cumulation of effects. Details of experimental data and the general biological significance of these findings will be discussed.

Glutathione content of blood following ingestion of acetone, section of the splanchnics, pancreatectomy, and adrenalectomy. M. M. KUNDE, T. J. BECKER (by invitation), F. S. SARUK (by invitation) and R. D. KEARNEY (by invitation). The Chicago Medical School, Department of Physiology and Pharmacology.

The blood glutathione of dogs shows a marked increase following the ingestion of acetone; removal of the adrenal and removal of the pancreas. There is a decrease in the glutathione content of the blood in the terminal stages of pancreatic diabetes and after bilateral section of the splanchnics. Section of one splanchnic causes no change.

The effect of the pituitary on carbohydrate metabolism. R. L. KUTZ (by invitation), H. SELYE (by invitation), O. DENSTEDT (by invitation), C. BACHMAN (by invitation), D. L. THOMSON and J. B. COLLIP. McGill University, Montreal.

In agreement with the work of Houssay it has been found that removal of the pituitary alleviates, at least partially, the symptoms which follow pancreatectomy in dogs. The transbuccal technic for hypophysectomy in dogs and its application to monkeys will also be discussed.

The influence of muscle sense fibres on the fatigue of the muscle. R. M. LEICK (by invitation) and F. H. SCOTT. Department of Physiology, University of Minnesota.

While the most obvious sign of fatigue in a person is a sensation, we can find no one who has studied the effect of the sensory fibres from the muscle in fatigue. We have experimented chiefly on frogs. The simplest plan of the experiment is, in animals under light urethane, to section one sciatic nerve leaving the other one intact and to stimulate both gastrocnemius muscles directly at about once a second. A faradic current was used and the contractions recorded on a slowly moving drum.

Under the above conditions it is easy to show that the muscle whose nerve is intact ceases to respond sooner than the one whose nerve is sectioned. This is true whether the muscles are supplied with blood or whether the blood supply to both muscles has been cut off. The reflexes from the muscle tend to accelerate the onset of fatigue (probably a protective mechanism—preventing the muscle from getting over fatigued).

The sensory fibres from one muscle seem to be connected with both sides since after section of the posterior roots on one side both muscles cease to respond at approximately the same time. Likewise, if the posterior roots are sectioned on both sides and the anterior roots on one side both sides cease to respond at approximately the same time. When, however, the dorsal roots of one side and the ventral roots of the other side are sectioned the leg whose ventral roots are intact is the first to show fatigue. Most if not all of the reflex effects are apparently sympathetic for if the rami going to the nerves on one side are sectioned the side with intact rami fatigues first. If, however, the rami on one side and the entire nerve on the other side are sectioned, then both sides fatigue at approximately the same time. These results might indicate the production of acetyl choline or similar substance. All the results mentioned occur whether the blood supply to the muscle is intact or whether it has been shut off.

Synergistic and antagonistic effects of milk and meat extracts. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, and Department of Physiology, Yeshiva College, New York. (Read by title.)

Cushny's old definition of pharmacology as "the study of the changes induced in living organisms by the administration in a state of minute

division of such organized substances as do not act merely as foods" is somewhat obsolete. We now know that such drugs as alcohol may act as foods while other substances commonly regarded as foods, e.g., vitamins, sugars, etc., may act as poisons. Considering the importance of synergistic phenomena, the author studied the pharmacological effects of milk and meat extracts, separately and in combination, on living animals and plants. The foods employed were: cow's milk, solutions of Valentine's beef juice, broths of beef muscle, chicken muscle and fish muscle, respectively, and emulsion of myosin made from fresh muscle by grinding and extraction without heat. The various solutions were first studied individually and then in combination on 1, *Lupinus albus* seedlings; 2, rats and mice after intraperitoneal injection; 3, larvae of *Rana sylvatica*; 4, *Carassius auratus*, and 5, kidney and liver function and blood chemistry of rabbits. *Phytopharmacologically*, one to five per cent milk solutions in plant-physiological saline were little toxic, while one to two per cent solutions of meat extracts were very toxic for seedlings, beef broth being more poisonous than chicken broth, and chicken broth more toxic than fish muscle extract. Combinations of milk and meat extracts produced marked potentiation of toxicity. Similar synergistic phenomena were noted in *animal* experiments. Milk and meat combinations, injected intraperitoneally, were more toxic for mice and rats than double quantities of either injected alone. Similar potentiation of toxicity was noted in goldfish and tadpoles placed in such mixtures. Two different sets of rabbit experiments were performed. On alternate days one set of rabbits received by stomach 100 cc. of whole milk and 100 cc. of meat extract for two or three weeks; the other set of animals daily received 50 cc. of milk and 50 cc. of meat extract together. Synergistic phenomena were noted in the rabbits receiving the mixtures.

Synergistic and antagonistic effects of alcohol, caffeine and nicotine mixtures.

DAVID I. MACHT and MARY E. DAVIS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore. (Read by title.)

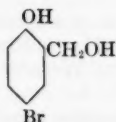
Phytopharmacological experiments were made on seedlings of *Lupinus albus* with solutions of ethyl alcohol, 0.5 to 1.0 per cent, of caffeine, 1:2,000 to 1:1,000, and of nicotine alkaloid, 1:10,000 to 1:5,000. After the toxicity of each separate drug had been determined, combinations of different pairs were studied and yielded the following results. A mixture of alcohol and caffeine produced synergism or increased toxicity for plants. Combinations of alcohol and nicotine also exerted an increased toxicity or synergistic action for plants while a mixture of caffeine and nicotine exhibited an additive effect and no potentiation. Zoöpharmacological experiments were performed on cats under ether anesthesia with caffeine solutions ranging from 1:500 to 1:200, nicotine alkaloid solutions ranging from 1:10,000 to 1:5,000, and ethyl alcohol solutions ranging from 0.5 to 2.0 per cent. After the lethal dosage of caffeine and nicotine alone had been determined by repeated injections at regular intervals, mixtures of the drugs were studied and their lethal dosage established. It was discovered that a combination of alcohol and caffeine produced an antagonistic action and that the toxicity of caffeine in this mixture was decreased. A mixture of alcohol and nicotine also exhibited a definitely antagonistic action, the amount of nicotine required to kill in such a combination being

greater than that required when it was administered alone. A combination of caffeine and nicotine also exerted an antagonistic action, the toxicity of the nicotine in this mixture being definitely decreased.

Physiological effects of three isomeric saligenins and their bromine derivatives.

DAVID I. MACHT and H. A. B. DUNNING, JR. (by invitation). Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore.

Three isomeric hydroxy-benzyl alcohols were prepared with the OH and CH₂OH groups in the ortho, meta and para positions, respectively, and a mono-brom derivative was made from each. A pharmacodynamic comparison of two sets of compounds was made on 1, *Lupinus albus* seedlings; 2, *Carassius auratus*; 3, rats in a maze; 4, mice; 5, circulation and respiration of cats; 6, kidney function of rabbits; 7, smooth muscle of the uterus and intestines, and 8, concerning their local anesthetic effect. In general, the ortho were more potent than the meta and the meta more potent than the para compounds. All the mono-brom derivatives were more active than the corresponding hydroxy-benzyl alcohols or saligenins themselves. The brominated compounds produced antipyresis. They also produced more sedation of the central nervous system and antagonized the convulsant action of camphor, the latter effect being probably due to the bromination. All the compounds effected a lowering of blood pressure through peripheral vasodilatation. Twenty-five to fifty milligrams per kilo weight of cats, injected intravenously, did not impair the respiration. Large doses depressed the respiratory center. All the compounds produced local anesthesia and relaxed smooth muscle. The most interesting of these compounds is mono-brom-ortho-hydroxy-benzyl alcohol, named Brom-salizol. It is little toxic, the minimal lethal intravenous dose being

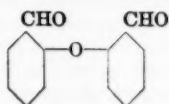


0.25 gram for cats and dogs and the minimal lethal dose given by stomach tube being 0.5 gram for rabbits. Men tolerate doses of from 5 to 10 grains taken three or four times daily with impunity. The most striking physiological effects of small doses in animals and men are a local anesthesia (a 0.5 per cent aqueous solution being sufficient for infiltration anesthesia in general surgery) and an antispasmodic action on smooth muscle organs both in vitro and in vivo. The compound has been used successfully for relief of spasm, excessive peristalsis and colicky pains of genito-urinary and gastro-intestinal organs. A third physiological effect now being investigated is the potentiated salicyl action of Bromsalizol as an analgesic and antiarthritic.

Physiological action of disalicyl aldehyde. DAVID I. MACHT and NOEL FOSS (by invitation). Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore. (Read by title.)

This compound (Perkins, Ann., 145, 299) is a beautiful white crystal-

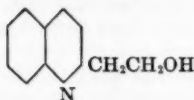
line substance with a melting point of 129–130° but, unfortunately for biological experimentation, it is insoluble in water and alcohol and only



soluble in acetone. Experiments were made by administering the drug in aqueous suspension to rabbits by stomach tube. Although insoluble in water, the compound doubtless undergoes a change in the body because doses of 0.5 gram per kilo produced death of rabbit in twenty-four hours. Small doses, 100 to 200 mgm., administered daily for some time, produced marked emaciation and a cachectic state. The drug also produces marked constipation. In normal animals no antipyretic effect was noted but in hyperpyretic rabbits a distinct drop in temperature was produced by 200 mgm. given by mouth. Urine collected one, two and five hours after oral administration showed that the drug had produced no bactericidal or bacteriostatic action. There was no impairment of kidney function and no narcotic or hypnotic effect.

Physiological effects of quinolyl-ethanol. DAVID I. MACHT and NOEL FOSS (by invitation). Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore. (Read by title.)

This compound, prepared by Wilhelm Koenig's method (*B* 32, 223), is a white crystalline powder with a melting point of 100–101°, slightly soluble



in water and freely soluble in alcohol. Experiments on *Lupinus albus* seedlings with solutions of 1:10,000 in one per cent alcohol produced marked inhibition of growth, the phytotoxic index being 37 per cent. Goldfish placed in concentrations of 1:5,000 in one per cent alcohol succumbed in five hours. One milligram per 50 grams weight of rats, injected intraperitoneally, produced little effect. Two hundred milligrams, administered by stomach tube to rabbits, produced no toxic symptoms and no impairment of kidney function. Intravenous injection of weak solutions in cats under ether anesthesia produced marked fall in blood pressure but little depression of respiration. Compared with phenmethylo, the compound is much less effective as an antispasmodic for smooth muscle preparations and has practically no local anesthetic action.

Detoxification experiments on pemphigus blood. DAVID I. MACHT and ISAAC R. PELS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore. (Read by title.)

The authors have studied over fifteen hundred specimens of blood sera from all kinds of dermatological conditions by Macht's phytopharmacological methods, described elsewhere, on living seedlings of *Lupinus albus*. The vast majority of sera from dermatological diseases produce a normal

phytopharmacological reaction, the same as that obtained with sera from normal individuals. Blood from the infectious exanthemata, scarlet fever, measles, variola and varicella, gives a less toxic reaction than normal blood. Characteristic phytotoxic results, indicated by marked inhibition of growth of roots, are produced by two grave dermatological diseases, pemphigus and leprosy. Blood serum and serum from the bullae of pemphigus exerted on seedlings a characteristic toxic effect which has been found invaluable in establishing diagnosis of pemphigus and differentiating it from other dermatoses simulating it, particularly erythema multiforme and dermatitis herpetiformis. The authors have studied the effects of physical and chemical agents *in vitro* in an endeavor to detoxify pemphigus serum. Heat above 75°F. produces rapid loss of toxicity which is not due to putrefaction. Exposure to ultraviolet rays does not detoxify pemphigus serum. A number of drugs and dyes, added to the serum, produced no appreciable detoxification. Ergosterol, recommended therapeutically by some clinicians, effects but little change in the phytotoxic index. The most interesting results were obtained with methylthionine chloride. Even very weak solutions of methylene blue (1:100,000, or less) markedly detoxified the serum, as studied on *Lupinus albus* seedlings. A chemically pure and biologically assayed solution of methylene blue, 0.5 per cent, has been prepared for cautious therapeutic use by intravenous injection in treatment of pemphigus, usually an incurable disease. In three clinical cases, a series of injections, 10 cc. each, given several times a week, produced amelioration of subjective symptoms, disappearance of skin lesions and marked improvement in the phytotoxic index. In two other cases, phytopharmacological examination has revealed distinct detoxification of blood and there has also been some amelioration of subjective symptoms, but the lesions have not disappeared.

The augmenting effect of the chorda tympani on sympathetic salivary secretion after atropine. F. C. MACINTOSH and H. C. RAWLINSON (by invitation). Departments of Physiology and Histology, McGill University.

The augmenting effect of chorda tympani stimulation upon the amount of saliva secreted by the cat's submaxillary gland in response to stimulation of the cervical sympathetic nerve is well known. It has been stated that this chorda augmenting effect is not apparent after administration of atropine. A series of experiments has shown that, when very small doses ($\frac{1}{4}$ to $\frac{1}{2}$ mgm.) of atropine are given intravenously, the secretory response to chorda tympani stimulation is, as usual, completely abolished, but such stimulation still causes a markedly increased secretion in response to subsequent sympathetic stimulation. This increased response is usually obtained, though in decreasing degree, in the subsequent two or three purely sympathetic stimulations.

When adrenalin was injected into such a lightly atropinized animal, its secretory effect could be prolonged by chorda stimulation, or if such stimulation was applied just at the end of the adrenalin response, a further secretion occurred.

Gastrectomy and subsequent hematologic studies in the hog. G. L. MAISON (by invitation) and A. C. IVY. Departments of Physiology and Pharmacology, Northwestern University Medical School.

A first series of 4 pigs was operated June 24, 1933, in the Physiology Laboratory of the University of Wisconsin Medical School. Of these, 1 died immediately and a second after 71 days due to fibrous stricture of the lower esophagus. The remaining 2 are living and well on their 225th post-operative day. A control pig has been kept with these and all are now 11 months old. On February 1, 1934, a second series of four animals was operated with an immediate mortality of 25 per cent.

Three of the first series developed a hypochromic microcytic anemia during their second month which became progressively worse in the two which survived into the third month. In the fifth month, iron ammonium citrate in large doses (av. 2.4 grams per day) was given in the food, but the anemia became progressively worse. Therefore, in the sixth post-operative month, subcutaneous Fe_2NH_4 citrate was started. A prompt hematopoietic response was obtained and at the present time after two and one-third months on this treatment, the hemoglobin has returned to the normal range as has the cell diameter and hematocrit.

The growth of the gastrectomized pigs was markedly retarded but has improved since the institution of the subcutaneous iron therapy though it is still not normal.

Summary. In 2 gastrectomized pigs we have observed the occurrence of a microcytic hypochromic anemia which started two months post-operatively and did not respond to 2.4 grams of iron salt per day orally, but did respond to 0.09 gram Fe_2NH_4 citrate daily subcutaneously. Gastrectomy markedly retarded the growth of these animals.

Bioelectric potentials and velocity of cell oxidation. GORDON MARSH (by invitation). Zoological Laboratory, State University of Iowa.

A complete connection is derived between the velocity of cell oxidation at flux equilibrium and the measured potential of a living cell or tissue upon the basis of the (amended) steps in the oxidative reaction proposed by Lund. The production of oxidizable substance (reductant) from its precursor is shown to be reversible, and the conversion of reductant to oxidant assumed to be dependent upon oxygen pressure. The potential at a single locus in the cell becomes that of a modified oxygen electrode and can be expressed as a function of the oxygen pressure of the medium and the velocity constants of the respiratory reactions. The potential increases with increased oxygen pressure.

Upon the assumption of some fundamental difference in the velocity of the oxidative reaction at the two ends of a cell (to account for cell polarity) the system will explain the dependence upon oxygen pressure of the E.M.F. measured across the cell. A relation is derived between the oxygen consumption of a cell at constant oxygen pressure and its inherent E.M.F.; the higher the rate of oxygen uptake (higher concentration of oxidizable material) the greater will be the E.M.F. This gives a theoretical foundation to the observed coincidence of high potential and high rate of oxygen consumption in polar tissues. It is shown that under appropriate conditions the cell having the higher rate of oxygen consumption will show the greater increase in inherent potential with increased oxygen pressure in accordance with experimental fact. The system will also account for reversal of cell or tissue polarity with lowered oxygen pressure.

The velocity equations at a locus within the cell may be synthesized into a theoretical equation linking the rate of oxygen consumption of a cell

or tissue to the oxygen pressure of the medium. This equation provides a satisfactory description of a variety of published experimental data, and possesses some points of advantage over the hyperbolic form of equation proposed by Warburg, Gerard and others.

The effect of certain metabolic products on tissue metabolism at normal and febrile temperatures. M. ELIZABETH MARSH. Department of Vital Economics, University of Rochester Medical School, Rochester, N. Y.

A Warburg apparatus was used to determine the effect of various metabolic substances upon the oxygen consumption of liver tissue. Urea, uric acid, histamine and phenol have been examined and in each case a certain concentration, varying with the substances used, would definitely depress the metabolism at normal temperature (37.5°C.) but would stimulate slightly the oxygen consumption of tissue from the same organ when kept at a febrile temperature (41°C.). This inhibition was, in some cases, fairly constant over the whole period of the observation, usually three hours, but in others it was a progressive one. The stimulation, on the other hand, was usually of short duration and during the second and third hours of the observation the oxygen consumption might fall much more rapidly than did that of the controls in Ringer's phosphate glucose solution at 41°C. and much more rapidly than did the tissue to which the substance had been added but which had been maintained at 37.5°C.

Oxygen absorption through the skin. BARTGIS MCGLONE, SAMUEL GOLDSCHMIDT and JOHN S. DONAL, JR. (by invitation). University of Pennsylvania, School of Medicine.

The following observations constitute convincing evidence that oxygen may penetrate the skin of the forearm into the blood of the cutaneous vessels.

When the circulation is partially or completely occluded:

1. The normal color of the skin is maintained when the arm, in an atmosphere of oxygen, contains its usual amount of blood. The cyanosis which usually develops on the skin of an arm in air with arrested or impeded circulation is not in evidence except on some portions of the hand.

2. The skin of the forearm, congested with blood, in oxygen, is pink; in contrast to the bluish or dusky color of an arm in air, nitrogen or hydrogen under like conditions.

3. Under the above experimental conditions the skin color may be altered from normal to cyanotic or from pink to blue or vice versa by changing the gaseous atmosphere appropriately.

4. The oxygen content of the cutaneous blood of a forearm, in an atmosphere of oxygen, is invariably higher than it is when the arm is in nitrogen.

Venous pressures and posture in normal young women. JOSEPHINE M. MCINTIRE (by invitation) and ABBY H. TURNER. Physiological Laboratory, Mount Holyoke College.

Determinations of venous pressure were made in a series of seven normal, healthy, young women of college age, reclining and tilted to erect and semi-erect positions, to test both the steadiness and the variability of venous pressure during these postural changes. The venous pressures determined were those in the superficial veins of the hand and foot, obtained by the indirect method of Krogh, Turner and Landis. In the super-

ficial hand veins at heart level a constancy of venous pressure was noted for each individual from time to time, and, contrary to the findings of some others, this constancy was maintained in spite of changes in posture. Steady experimental conditions were absolutely necessary and careful precautions were taken to prevent any obstruction to the return of blood to the heart. The increase in venous pressure found in the foot on assuming the erect position was great enough to assure an adequate return of blood to the heart. In one case in which circulatory adjustment to standing was known to be habitually poor venous pressure in the foot vein was not adequate in the erect position to return blood to the heart.

The effects of posterior-lobe pituitary preparations upon the concentrations of copper-reducing substances in the serum and urine of dogs. A. R. MCINTYRE. Pharmacology Laboratory, University of Nebraska Medical College, Omaha, Nebraska.

The serum copper-reducing substance of normal unanesthetized dogs fasted 18 hours was invariably augmented in concentration as the result of the subcutaneous injection of pituitrin, pitressin, and pitocin either separately or together. The effects of these preparations upon the concentrations of the urine copper-reducing substances were not constant and frequently the urine copper-reducing substances were decreased in concentration following the subcutaneous injection of 0.47 to 1.0 unit per kilogram. Thus in 12 experiments in which pituitrin caused an average increase of the fasting blood sugar of approximately 14 per cent there was an accompanying decrease in concentration of the urine copper-reducing substances in 4 experiments and in the remaining 8 experiments there was an increase comparable with that found in the serum. Very similar results were observed with pitressin. With pitocin the urine copper-reducing substances were decreased in concentration only twice in 12 experiments. These decreases in concentration of the urine copper-reducing substances cannot be attributed wholly to urine dilution during diuresis as the increases in concentration were also observed during diuresis and furthermore it has been previously shown (McIntyre and Sievers, *Journ. Pharm. Exp. Therap.*, **49**, 1933) that a decrease in the concentration of the urine copper-reducing substances may occur simultaneously with a greatly augmented concentration of K, Na, Ca, and Cl. The Harding and Selby fermentation technique indicated that much of the urine copper-reducing substance was non-fermentable. There did not appear to be any constant relationship between the proportion of fermentable and non-fermentable substances in the urine either before or after the injection of the above preparations.

The cells of an inflammatory exudate in relation to its hydrogen ion concentration. VALY MENKIN. Department of Pathology, Harvard Medical School, Boston, Massachusetts. (Read by title.)

A pleural inflammatory exudate, in the majority of instances, develops a rise in its hydrogen ion concentration concomitantly with the progress of the inflammatory reaction. When the pH of the exudate is alkaline the percentage of polymorphonuclears at the site of inflammation exceeds that of the mononuclear phagocytic cells. When the pH of the exudate is approximately neutral the percentage of polymorphonuclear cells tends to approach that of the mononuclear phagocytes. When the pH of the

exudate is definitely acid large numbers of polymorphonuclear cells are found degenerated. The percentage of relatively normal appearing polymorphonuclear leucocytes is found considerably lower than that of the mononuclear phagocytes. In some cases the pH of the exudate remains alkaline throughout the period of an acute pleural inflammation. In these instances the percentage of polymorphonuclears invariably exceeds that of the mononuclears. By measuring the hydrogen ion concentration of an inflammatory exudate the character of the cytological picture can be predicted with a fair degree of certainty. Likewise the converse follows. Evidence has been obtained to show that the development of a local acidosis in an area of inflammation precedes, at times, the changes occurring in the differential leucocyte formula of the exudate. In such cases, however, the cytological changes ultimately follow the development of the acid reaction.

The observations reported suggest that the differential leucocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytological picture in an inflamed area seems to be conditioned by the pH of the exudate surrounding the injured tissue. The present study indicates that the developing local acidosis, as the inflammatory reaction progresses, can adequately account for the well known shift in infiltration from polymorphonuclear leucocytes to mononuclear phagocytes at the site of inflammation.

A v-phone for examining auditory impairment. W. R. MILES. Yale Institute of Human Relations. (Demonstration.)

A pair of earpieces of stethoscope type is connected with a 3-way glass stopcock from the median portion of which extends a rubber tube 24 inches in length and terminating in a small metal funnel. Sound led through this central rubber tube can be presented to both ears or exclusively to the right or left, or cut off from both ears accordingly as the stopcock is turned. With the v-phone so placed that the stopcock is at the back of the patient's head and resting between his shoulders the examiner may secretly change the presentation.

A metabolic study of three unusual learned breathing patterns practiced in the cult of Yoga. W. R. MILES and K. T. BEHANAN (by invitation). Yale Institute of Human Relations.

Swimmers, divers and others temporarily modify the usual breathing pattern for occupational reasons. In connection with the rigorous religious regimen of Yoga, dating from the third century B.C., extreme variations from ordinary breathing habits are also learned and used daily for periods of from one to several hours. A study of these extreme variations is of great theoretical interest. The present report concerns three of the nine Yogic breathing forms. The subject was one of the writers, K. T. B., a Hindu male, 31 yrs., height 164 cm., weight 57 kgm., who had lived on a vegetarian diet and engaged in almost daily practice of these breathing patterns for 20 months, having spent 12 of these months under the instruction of a Yoga teacher of note in India.

The three exercises may be described as follows: I—Kapalabhati, 120 very short breaths in 60 seconds followed by natural breathing for 30 to 60 seconds, cycle repeated several times in succession. II—Ujjayi, extreme deep inspiration, holding for 40 to 80 seconds then slow expiration, cycle

repeated perhaps 80 times. III—Bastrika, 20 very short breaths in 10 to 12 seconds followed by extreme deep inspiration, holding for 30 to 60 seconds, then slow expiration, cycle repeated perhaps 80 times.

The subject was always studied under basal metabolic conditions. When reclining he showed among other measurements: oxygen consumption of 210 cc. per kgm. per hour, respiratory rate 17.3 per minute, ventilation rate 7.6 l. per minute, and vital capacity 3.3 l. When seated with legs crossed and clothed as prescribed for the practices the normal oxygen consumption was 220 cc. with a respiration rate of 17.7. In breathing patterns I, II, and III the oxygen rates were 293, 298, and 286 cc. respectively averaging about 13 per cent increase. Six cycles of I required 10 minutes and the short breaths measured 0.2 to 0.3 l. In II the full vital capacity was regularly employed, 67 per cent of the total time was devoted to holding the breath, and 9 total cycles required 8 minutes. Also in III the full vital capacity, 3.3 l., was used, 6 cycles occupied 8 minutes and holding accounted for 58 per cent of the time.

Summarizing: the three unusual breathing patterns were found to involve periods of very rapid shallow respiration, the repeated use of full vital capacity, and of long periods of breath holding. Each combination, I, II, and III, continued for 10 minutes or longer produced a moderate increase in the oxygen consumption. This effect is probably due chiefly to the increased physical work performed by the respiratory muscles in carrying out the exercises. Continuance of the exercises for 20 to 30 minutes did not, during the next 10 minutes, with the exception of one experiment with II, develop evidence of oxygen debit or storage. The investigation is being continued. Slides accompany the paper.

Effect of glycocoll and ephedrine in myasthenia gravis. A. T. MILHORAT (by invitation). Russell Sage Institute of Pathology, in affiliation with the New York Hospital and the Department of Medicine, Cornell University Medical College.

Patients with myasthenia gravis usually show a moderate creatinuria and an impaired ability to retain ingested creatine. The administration of glycocoll often increases the creatine excretion, or is followed by a creatinuria where none previously existed. When creatine is administered after the patient has been receiving glycocoll for a few days the retention is less than when creatine is given alone. A similar effect of glycocoll is observed in other types of muscle disease, and therefore is in no way specific for this condition. However, the absence of such an effect is considered as evidence against the diagnosis of myasthenia gravis. The administration of ephedrine is without effect on the creatinuria, the creatine tolerance, or the action of glycocoll in altering the creatine tolerance. Both glycocoll and ephedrine are useful in the therapy of myasthenia gravis, and when both are administered together, a summation of their effects is obtained. Patients who had shown clinical improvement for many months when given glycocoll or ephedrine alone, displayed additional improvement when both were given together.

Though ephedrine and glycocoll both have therapeutic effects, their action is different. To afford an explanation for these observations it is postulated that glycocoll acts primarily in furthering synthesis of phosphagen, whereas ephedrine probably increases the rate of phosphagen resynthesis without affecting the urinary creatine.

Determinations of the pH of glomerular and tubular fluid in frogs and necturi.

HUGH MONTGOMERY and J. A. PIERCE (by invitation). Laboratory of Pharmacology, University of Pennsylvania.

Two methods have been developed for determining pH in minute amounts of fluid. One, colorimetric, makes use of phenol red in capillary tubes. The other, devised by Pierce in the laboratories of the Pediatrics Division of the Johns Hopkins Hospital, is a micro adaptation of the quinhydrone electrode. Both have been applied to the reactions of fluid in glomeruli and tubules. The results show that glomerular urine and plasma have the same pH; that no significant change occurs during passage through the proximal tubule. The fluid becomes acid in the distal tubule.

Reactions to irritating arterial injections in decerebrate and chronic spinal cats. R. M. MOORE and E. L. PORTER. The University of Texas, Medical Branch, Galveston.

One of us has previously described the effects of intra-arterial injections of irritating solutions in cats anesthetized with sodium-amytal or with dial. This study has been extended by injections into the femoral arteries of animals in the acute decerebrate state (Sherrington guillotine) and in the chronic spinal state 6 to 10 days after section of the lower dorsal cord. In the decerebrate preparation movements of the extremities and changes in respiration were considered indicative of stimulation of receptors for pain. In the chronic spinal animal the criterion was movement of the contra-lateral leg, usually extension, or movement of the tail. Sodium chloride provided stimulation when the concentration reached 2.0 to 3.0 per cent. Potassium chloride, made isotonic by adding sodium chloride, became effective at concentrations varying from 0.2 to 0.6 per cent. Mixtures of sodium hydroxide and acetic acid, made isotonic by addition of sodium chloride, were tested to determine the degree of acidity required for stimulation. Reflex effects occurred when the hydrogen ion reached the concentration represented by pH 6.0. Excepting obviously defective animals in which the values were high, these thresholds for stimulation in decerebrate and spinal cats are approximately the same as those for animals under sodium-amytal or dial. It is concluded that, despite the absence of vocalization, decerebrate and chronic spinal animals may be used profitably in studies of pain-sensibility.

Partitioning of phosphate compounds in blood during glycolysis. S.

MORGULIS. Department of Biochemistry, University of Nebraska.
(Read by title.)

The orthophosphate, pyrophosphate and hexosemonophosphate fractions of the total acid soluble phosphate compounds of the red blood cells have been determined before and after glycolysis in several animals, and an attempt has been made to correlate these findings with the degree of their glycolytic activity. In hog corpuscles, having a low glycolytic function, there is a very high content of P as orthophosphate and pyrophosphate, which increase and decrease respectively during glycolysis. The former may increase 200 to 300 per cent. The hexosemonophosphate also invariably decreases, as does the undetermined P fraction. However, no relationship to the glycolytic activity can be deduced from these changes. The condition in the poorly glycolysing beef red cells is essen-

tially similar. The total acid soluble P being less than half the amount found in the hog cells the absolute differences are not so striking, but there is the same reciprocal relation between the changes in the orthophosphate and pyrophosphate fractions, only the diminution in the hexosephosphate or in the undetermined P fraction occurs here very exceptionally. In a third type of poorly glycolysing cells, those of the horse, we still observe the striking rise in inorganic P during glycolysis, but the pyrophosphate P remains practically unchanged. The hexosemonophosphate, however, shows a greater or less diminution corresponding more or less to the glycolytic activity.

Two strongly glycolysing systems were also studied, rabbit and dog red blood cells. The acid soluble P concentration of the rabbit cells is very similar to that of the hog blood cell, and the changes which one observes in the inorganic, pyrophosphate and hexosemonophosphate P fractions during glycolysis are very similar in spite of the very great difference in the glycolytic activity of these two types of cells. However, considerable decreases in the undetermined P fraction are found in rabbit blood cells which do not seem to be directly related to their glycolytic activity. In glycolysing dog cells the inorganic P fraction may increase five- or sixfold, but the changes in the pyrophosphate fraction are variable; both increases and decreases have been found. The hexosemonophosphate fraction as a rule decreases, as does also the undetermined P fraction, but no relation can be established between these changes and their glycolysis.

The effect of alcohol on cutaneous tactile and pain sensitivity. F. J. MULLIN (by invitation) and ARNO B. LUCKHARDT. Physiological Laboratory of the University of Chicago.

Using the von Frey hair technique we studied the effect of the ingestion of 300 and 375 cc. doses of 20 per cent alcohol on cutaneous pain and tactile sensitivity. Control determinations were made on eleven skin spots on the face and hand regions immediately before the alcohol was taken. The same spots were used for obtaining readings of pain and tactile sensitivity every half-hour for $2\frac{1}{2}$ to $3\frac{1}{2}$ hours following the ingestion of the drug. Twenty-five experiments were performed on a total of ten persons. In five of the subjects blood and urine samples were collected for the purpose of analysing the alcohol content at the time of each sensitivity determination.

In every case the alcohol caused a distinct decrease in the pain sensitivity lasting about two to three hours. There was always a marked dissociation between the tactile and pain senses. The alcohol had practically no effect on the sensitiveness to touch as measured by this method. The general subjective sensations of the person tested did not parallel exactly the changes in the objective touch and pain responses following these amounts of alcohol. Nor was there a strict correlation between the above factors and the alcoholic content of the blood, both the general subjective feelings and the response to peripheral stimulation returning to normal before the blood alcohol had dropped to the control level. A short-lasting period of increased sensitivity to pain was usually recorded immediately following the depression caused by alcohol.

The larger dose of alcohol gave more marked changes in sensitivity than the 300 cc. dose, though both doses caused a depression in sensitiveness to pain as definite as, or more so, than that caused by corresponding doses of

other analgesic drugs (morphine sulphate, aspirin, codeine sulphate, acetanilid, antipyrine, pyramidon) or by sodium bromide or phenobarbital.

Use of the von Frey hairs for studying cutaneous sensitivity. F. J. MULLIN (by invitation) and ARNO B. LUCKHARDT. Physiological Laboratory of the University of Chicago. (Demonstration.)

In order better to acquaint the members of the Federation with the technique referred to in a communication on the effect of alcohol on cutaneous sensitivity, a set of von Frey hairs for determining tactile and pain sensitivity will be demonstrated. Charts and typical results of experiments obtained by the use of this method will also be shown.

A semi-automatic respiration calorimeter for man. JOHN R. MURLIN and ALAN C. BURTON (by invitation). Department of Vital Economics, University of Rochester, Rochester, N. Y.

The instrument was designed originally for application of high frequency currents to the human subject and study of the effects of hyperthermia induced in this way. Consequently it is constructed largely of glass, wood and other low-conducting materials. The inner chamber wall is composed of pyrex glass in the form of 4 flanged cylinders measuring 23 inches in diameter, 19 inches in length and $\frac{1}{2}$ inch thick. The cylinders are held together by bakelite rods and bakelite collars which compress rubber gaskets between the flanges.

The water grid for absorption of heat is also composed of pyrex glass. Water circulates through this grid under a constant head and regulated thermostatically as to initial temperature. The difference of water temperature between the points of entering and leaving the chamber is registered automatically on a thread recorder every two minutes, from resistance thermometers connected into a wheatstone bridge. The water rate is measured with an automatic water meter. Heat lost or gained to the chamber by the ventilating air current is recorded automatically in similar manner to the water current, i.e., an air-flow meter and resistance thermometers. The calorimeter is not adiabatic but emissive, some heat loss through the wall being purposely permitted, and measured. After trial of several methods the one which has proved most satisfactory is the use of two long nickel resistance wires sewed into tapes which are wound spirally about the outside of the glass chamber and on the outside of a blanket of insulating material ("Dry Zero"), on top (outside of) the glass chamber. The difference of resistance in these two wires recorded automatically serves to establish gradients of temperature across the insulation for different rates of heat production inside the chamber. By previous calibration with accurately known rates of heat production by electrical generation, corresponding to a given gradient, the heat loss through the wall from the human subject can be measured to an accuracy of five per cent.

The ventilating air circuit is provided by a blower and the air circulates through a Benedict train for measurement of water and carbon dioxide, oxygen being admitted and measured through a meter. Examples of accuracy will be given.

The experimental production of ventricular fibrillation, and its prevention by β methyl acetyl choline chloride. L. H. NAHUM (by invitation) and H. E. HOFF. Laboratory of Physiology, Yale University.

Sudden death in acute experimental poisoning with benzol (C_6H_6) vapor results from ventricular fibrillation (Nahum and Hoff, Journ. Pharm. Exp. Therap., 1934). Within a minute or less after inhalation has begun ventricular extrasystoles appear from one or many foci, leading to a ventricular rhythm from a single focus. In cases of fatal poisoning the ventricular rhythm increases in rate, and becomes a true ventricular tachycardia which culminates in ventricular fibrillation.

Two factors have been shown to play a part in the production of these irregularities: a direct action of benzol on the myocardium, and the presence of adrenalin in the body. In animals from which the adrenal glands have been removed, and the stellate ganglia excised, inhalation of benzol does not produce ventricular extrasystoles, ventricular tachycardia, or ventricular fibrillations. Some or all of these rhythms promptly appear with the subcutaneous injection of adrenalin in quantities that before the inhalation of benzol produce none of these effects.

β methyl acetyl choline chloride (Mecholin) injected subcutaneously in doses of 2-5 mgm. per kilo, prevents the appearance of ventricular rhythms and thus protects the animals from sudden death. Quinine hydrochloride injected intramuscularly in whatever dose, had no effect on the appearance of these fatal rhythms.

The secretion of intestinal juice as influenced by certain drugs and extracts.

E. S. NASSET and H. B. PIERCE. Department of Vital Economics, University of Rochester, Rochester, N. Y.

Previous work in this laboratory has shown that dogs with 1st and 2nd stage intestinal transplants respond to a meal with an increased secretion of intestinal juice. Therefore, it was felt that a humoral mechanism had been demonstrated.

As indicated by Parry and Nasset (This Journal, **105**, 78, 1933) the technic developed by Wells (This Journal, **99**, 209, 1931) for the determination of the so-called "absorbing force" of the intestine is adaptable to the measurement of changes in intestinal secretion in acute experiments.

With the aid of this technic, the effects of intravenous administration of acid and salt extracts of the small gut of both dogs and swine were tried. These preparations were found to increase the rate of secretion and almost invariably to depress the blood pressure (carotid). In nearly all cases it was impossible to dissociate low blood pressure and increased secretion. The extracts contained enough secretin to stimulate the flow of pancreatic juice but the injection of purified secretin having a maximum effect upon the pancreas was without effect upon the small gut of the same animal.

Pilocarpine, physostigmine and epinephrine all gave positive results of short duration. Acetyl choline and histamine in sufficient amounts to give a lowering of blood pressure comparable to that obtained with the extracts failed to have as great an influence upon secretion.

The intravenous administration of 5 per cent Witte's peptone resulted in intermediate lowering of blood pressure and a great augmentation in the rate of secretion. Infusion of 4.5 grams of peptone in solution in one experiment resulted in an increase in the rate of secretion of 1 cc. per minute which continued for $\frac{1}{2}$ hour. Difco "Bacto-Peptone" failed to exhibit any effect upon either blood pressure or secretion. The administration of 50 grams of Witte's peptone by stomach tube to two dogs with chronic intestinal transplants was followed by a decided increase in the production

of intestinal juice. The effect was rather more pronounced in the dog in which the mesenteric connections to the transplants had been severed.

The secretion of mucus of the normal and diseased stomach. H. NECHELES and A. COYNE (by invitation). Department of Gastro-Intestinal Physiology of the Michael Reese Hospital.

A test for the mucus secreting function of the stomach was developed. Pilocarpin was injected subcutaneously and the stomach was evacuated through a tube by constant suction. Samples were taken every ten minutes and put into a refrigerator immediately. Four samples were taken before, and six after the injection of pilocarpin. The visible mucus was measured by centrifugating each whole sample in a graduated centrifuge tube, the dissolved mucus was precipitated with acetone. Saliva flowed abundantly after the pilocarpin, and was removed from the mouth by constant suction. Thirty-one normal persons and 22 patients with peptic ulcer were examined. Both, normals and patients must be grouped into two categories—alcoholics and non-alcoholics. The latter group includes persons drinking small amounts of alcohol occasionally.

Our results may be grouped as follows:

A normal person has about 9 per cent of mucus (percentage of visible mucus in the entire sample of stomach juice) in his fasting stomach. After pilocarpin it rises to 12 per cent. The corresponding amounts in the patients with peptic ulcer are 7 per cent and 6 per cent respectively. Habitual drinkers with peptic ulcer have 14 to 18 per cent respectively. Another noteworthy finding is that the combined acidity of the stomach juice rises after pilocarpin in the normals but not in the peptic ulcer patients. Mucus secretion seems to increase with age (the age of our normal subjects ranges between 18 and 54 years), but this must be confirmed on a larger series. We did not find a correlation between the amounts of dissolved and visible mucus.

Summary: In normal persons there is an increased secretion of mucus after injection of pilocarpin. This does not take place in patients with peptic ulcer. Drinkers may have peptic ulcer although their secretion of gastric mucus is greatly increased. It seems as if the mucous secretion of the stomach rises with age in normal persons.

The distribution of white blood cells in the peripheral circulation of emotionally excited rabbits. L. B. NICE and H. L. KATZ (by invitation). The Ohio State University.

The effect of excitement (anger, fear and pain) on the leucocyte picture in the peripheral blood (ear veins) in normal and splenectomized rabbits was studied. The basal counts by making a series of determinations at fifteen minute intervals were first established in rabbits deprived of food for twenty to twenty-four hours. The rabbits were then fastened back downwards to an animal holder and teased for approximately three minutes by being stimulated from time to time by a weak interrupted Faradic current. The counts were again taken at fifteen minute intervals until they returned to normal. Standardized Trenner pipettes were used and at least two hundred cells counted in the majority of cases.

In forty-six experiments on 17 normal rabbits, there was a marked leucopenia in forty cases or 87 per cent while four showed no change, and in two there was a slight leucocytosis.

In fifteen experiments on splenectomized rabbits there was a marked leucopenia in thirteen cases or 87 per cent, while there were negative results in two cases.

These results clearly indicate that emotional excitement in the rabbit is associated with a definite peripheral leucopenia which has no relation to the spleen. The leucopenia apparently is due to the redistribution of the white cells accompanying the vasoconstriction in excitement.

Effects of local cooling of the surface of the brain stem on respiration. HAYDEN C. NICHOLSON. Department of Physiology, University of Michigan, Ann Arbor.

A fine, copper tipped applicator through which fluid at varying temperatures could be circulated was placed in light contact with the surface of the brain stem. Most marked effects of cooling were obtained with the applicator in contact with the floor of the fourth ventricle only a few millimeters anterior to the calamus scriptorius. Varying the position of the applicator produced no distinct qualitative variation in effects. Definite changes in respiration could be produced with temperatures only ten or fifteen degrees below body temperature. More marked cooling might completely stop respiration. The usual effect of local cooling was a lengthening of the duration of the inspiratory phase of respiration at the expense of the expiratory phase. This usually resulted in a decrease in rate, sometimes very marked. However, in some cases in which respiration was characterized by a prolonged expiratory pause, cooling, by markedly shortening this pause might cause an increase in respiratory rate. These effects of local cooling could be obtained with either intact or sectioned vagi.

The effects on respiration of stimulation of the vagus and saphenous nerves during local cooling of the surface of the medulla were studied and compared with the normal effects. The inhibition of respiration normally produced by strong stimulation of the central end of the vagus was almost impossible to obtain during local cooling, current strengths which would normally produce it having no effect or causing an acceleration. On the other hand, the increased respiratory rate normally resulting from strong stimulation of the saphenous was more marked and more easily produced during local cooling of the surface of the medulla.

The relation of the time phases of muscular contraction in reciprocal innervation. T. L. PATTERSON. Wayne University, College of Medicine.

The general characteristics of the muscular manifestations accompanying reciprocal innervation have been described by Sherrington. In the present investigation a study was made of the simultaneous time phases of the muscular contraction of a protagonist coexisting with inhibition of its antagonist. The gastrocnemius and tibialis anticus muscles of the grass frog, *Rana pipiens*, were employed. These muscles were isolated and the rest of the leg below the knee joint was cut away, as in making a nerve-muscle preparation. The animal was then mounted on a frog board which was placed slightly in an oblique position and attached to an iron stand. The tendons of the two muscles were attached to pulley wheels of separate light Harvard muscle levers and the contractions were recorded on a wide and rapidly moving plate of a Lingle spring myograph. The animals were double pithed and single induction shocks were applied

directly to the muscle. The activity of the two muscles was obtained under the following conditions: 1. Stimulation of the gastrocnemius with sciatic nerve intact. 2. Stimulation of the gastrocnemius with sciatic nerve sectioned. 3. Stimulation of the tibialis anticus with sciatic nerve intact. 4. Stimulation of the tibialis anticus with sciatic nerve sectioned.

An analysis of the results indicates that the excitatory phase of the protagonist exists with a simultaneous inhibitory phase of the antagonist, which is then followed by a contraction during and just following the beginning of relaxation of the protagonist. Furthermore, reciprocal reaction of the two muscles could be reversed. The length of the latent periods of protagonist and antagonist muscles are in support of the reciprocal innervation theory of Sherrington, Stein and Tulgan, et al. However, Stein and Tulgan were unable to obtain a reciprocal reversal of these two muscles in either the frog or the cat, but with the flexor carpi ulnaris and the extensor carpi ulnaris, as well as the biceps and triceps of the cat, a reciprocal reversal could be elicited.

Increased plasma bilirubin following intravenous injection of isoideiken (phenol-tetraiod-phthalein-sodium). W. D. PAUL (by invitation), B. B. CLARK (by invitation), and F. M. SMITH. Laboratory of Pathological Chemistry and The Department of Internal Medicine, The State University of Iowa, Iowa City.

In reviewing the incidence of cholecystitis in patients with diabetes mellitus, it was found that in the majority the gall bladder failed to be visualized on x-ray. To further determine this point, a series of patients with diabetes were given iso-ideiken intravenously. Following the suggestion of Rosenthal, the liver function was also studied by determining the amount of dye in the blood plasma at definite intervals following the injection. In this procedure 1 cc. of blood plasma was precipitated by acid alcohol, centrifuged, the supernatant fluid made alkaline and allowed to stand for a few hours in the ice box, and again centrifuged. This gave a clear solution which could be compared in the colorimeter even when icteric bloods were used. It was noted that the blood plasmas drawn one-half hour or more after injection of the dye were more highly colored, and when a slight degree of icterus was present this seemed to be intensified. Bilirubin determinations (Gibson and Goodrich) were done routinely, therefore, on all specimens of blood drawn. These showed an increase in blood plasma bilirubin, reaching a maximum in 1½ hours after injection, and persisting for at least 24 hours. This increase was about 1 to 2.5 mgm. in non-icteric cases, and reached as high as 5 mgm. in jaundiced patients. Of 26 patients studied, all have shown an increase in plasma bilirubin. Three additional patients given the same dye by mouth showed increases of only 0.2 to 0.5 mgm. The results are interpreted as indicating actual injury to the liver caused by the dye injected (2.5 gm. in 30 cc. of distilled water).

✓ *Carbohydrate metabolism studies in hypophysectomized albino rats.* R. A. PHILLIPS and PHOEBE ROBB (by invitation). Collis P. Huntington Memorial Hospital, Boston, Mass.

Healthy, growing (70-140 grams) albino rats were hypophysectomized by the neck approach. Fifteen to sixty days later these animals and litter mate controls were fasted for 48 hours. They were then fed, by stomach

tube, 0.6 gram dextrose (in 30 per cent solution) per 100 grams body weight. One, 2, 3 and 4 hours later they were sacrificed after administering sodium amytal intraperitoneally. The content of glucose remaining in the gastrointestinal tract, the blood sugar and the liver and muscle glycogen values were then determined. Fasting values for the last 3 were also obtained.

After hypophysectomy intestinal absorption was about 30 per cent lower than in the controls. Blood sugar values were about the same after feeding though much lower in the fasting animals (35–50 mgm. per cent). Glycogen was stored at a much lower rate in liver and muscle, after hypophysectomy, particularly in the latter. The storage was much lower than could be accounted for on the basis of lessened intestinal absorption. Only 7 to 11 per cent of glucose absorbed in 4 hours was deposited as liver glycogen as compared with 18 per cent in controls. Since the actual mass of muscle mass in the rat after hypophysectomy is not known it was not possible to determine the amount of absorbed glucose stored as muscle glycogen. The average value of muscle glycogen 4 hours after feeding was 0.65 per cent in the controls while in the hypophysectomized rats it was 0.21 per cent.

Studies on the transplanted intestinal loop. III. The chemical analysis of intestinal juice. H. B. PIERCE and E. S. NASSET. Department of Vital Economics, University of Rochester, Rochester, N. Y.

Intestinal juice was collected from sections of the upper jejunum 15 to 20 cm. in length which had been transplanted under functionally hypertrophied mammary glands of dogs. Experiments were conducted both before and after division of the mesenteric pedicle. In the latter condition the collateral circulation developed from the mammary vessels was adequate to maintain the loops in good condition. Succus entericus was collected during fasting periods and periods following food.

Some analyses were made directly upon the fresh juice. In other experiments the juice was dried at 38 to 40°, and ground to a powder prior to analysis.

Solids, total nitrogen, ammonia, carbonate, chlorine and ash were determined in the fresh juice, and water, total nitrogen, urea, ammonia, ether extract, ash, chloride and carbonate in the dried juice.

The average content of solids of the fresh juice was 2.63 per cent. The results of a typical analysis on the mixed dried intestinal juice of several dogs are as follows: Water 4.93 per cent, total nitrogen 8.85 per cent, urea 1.28 per cent, ether extract 3.33 per cent, ash 29.94 per cent, chloride as NaCl 24.73 per cent and carbonate as Na_2CO_3 2.59 per cent.

The routes of absorption of caffeine and of strychnine in fish. F. H. PIKE. Columbia University. (Demonstration.)

Small marine fish, e.g., *Fundulus*, are placed in separate containers with 1, caffeine, and 2, strychnine in solution in sea water. The specimens in the caffeine solution swim about freely at first, but soon begin to show a rigidity of the muscles, leading to a constraint of movement and eventual death. The fish in the solution of strychnine swim about with no obvious symptoms so long as there are no particles in the sea water which they may swallow. They do not show any convulsions even after considerable intervals in the solution. If, however, a few crumbs are dropped into the solution, the fish swallow them and convulsions appear in a few minutes.

The inference is that caffeine may be absorbed through the buccal or branchial membranes while strychnine is not. Strychnine is, however, readily absorbed from the gastric mucosa.

A second inference is that, in a perfectly uniform medium touching the mucous membrane of the mouth, no excitation for swallowing is set up, for when the fish swallow crumbs, they soon develop convulsions. Some departure from the uniformity of conditions is a necessary condition for inducing swallowing.

Apparatus needed: One large glass dish, flat, 8 or 10 inches in diameter, 2 or 3 inches high. Three smaller dishes, 4 to 6 inches in diameter, 2 or 3 inches high. Two poison labels.

Space needed: About 4 square feet of flat desk or table space.

I will provide the fish, the caffeine, the strychnine, the sea water and the crumbs.

Technical assistance needed: none.

Distribution of phosphorus compounds in striated and cardiac muscles of the dog. HERBERT POLLACK, EUNICE FLOOK (by invitation) and JESSE L. BOLLMAN. Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota.

Analyses of striated and cardiac muscle of the dog were done to determine the distribution of the phosphorus-containing compounds under normal conditions and after various dietary managements. In normal striated muscle the total acid-soluble phosphorus varied from 114 to 142 mgm. in each 100 grams of tissue. Phospho-creatine values were more constant, varying from 34 mgm. to 44 mgm. in each one hundred grams of tissue. The quantity of the Embden ester present was quite constant, the values being from 5 mgm. to 9 mgm. in each 100 grams of muscle. The ortho inorganic phosphate phosphorus averaged around 30 mgm. The greatest variations observed were in the nucleotide phosphorus fraction. More specifically pyrophosphate had been removed by hydrolysis. This might indicate that adenosine is not the only free nucleotide in the muscle extract. The analysis of the cardiac muscle illustrated the dependence of creatine phosphate on an adequate oxygen supply. In removing hearts from intact animals artificial respiration must be instituted before opening the chest wall. The phosphocreatine content of the heart is about one-third of that found in an equal quality of striated muscle. The nucleotide fractions are as variable in the heart as in the striated muscle.

Bilateral integration of reptilian lymph hearts. F. H. PRATT and M. A. REID (by invitation). Boston University and the Evans Memorial.

Although the members of the multiple pairs of lymph hearts in Amphibia are synchronized homolaterally, there is as yet no evidence of bilateral association, automatic or reflex (Pratt and Reid, 1932; F. T. Brücke and Umrath, 1933). In various reptiles, however, it is found from mechanical graphic records that the single pelvic pair of lymph hearts are associated in their rhythm and often synchronized. Such conditions are conspicuous in the turtle, where there is both coördination of spontaneous beats and a highly developed system of local reflexes. Very light mechanical stimuli to the skin of flank or leg, passive movement of leg or tail, or mechanical or electrical stimuli to a lymph heart itself may elicit response of one or both hearts. Therefore the integration of spinal lymph-heart centers, purely

axial and homolateral in Amphibia, is in Reptilia, as exemplified in chelonians, subject to local reflexes influencing a system of crossed association.

The effect of the growth and thyreotropic hormones of the anterior pituitary upon the calcium metabolism of the rat. L. I. PUGSLEY and E. M. ANDERSON (by invitation). McGill University, Montreal.

Hypophysectomized rats on a low calcium diet showed a negative calcium balance, upon administration of the growth hormone. The calcium balance was changed to a positive one. This change in the calcium balance occurred with the resumption of growth.

An increase in the excretion of calcium in the feces was observed in normal rats upon administration of the thyreotropic hormone. This increased excretion coincided with the increase in basal metabolism.

The mechanism of gastric inhibition following ingestion of carbohydrates. J. P. QUIGLEY and K. R. PHELPS (by invitation). Department of Physiology, Western Reserve University Medical School, Cleveland, O.

The well-known inhibition of gastric hunger contractions which follows ingestion of carbohydrates may depend on nervous or humoral mechanisms. The absence of gastric inhibition following intravenous injections of lactose (Bulatao and Carlson) or dextrose (Quigley and Hallaran) tended to oppose the humoral explanation. However, the recent investigation of Quigley, Zettleman and Ivy on the humoral inhibition of the stomach by fats has prompted a similar investigation on possible humoral factors in gastric inhibition by carbohydrates.

Templeton and Quigley noted inhibition of a partly denervated stomach preparation (Heidenhain pouch) following dextrose feeding. We have observed inhibition of the completely denervated auto-transplanted gastric pouch and also the stomach denervated by vagotomy, splanchnicotomy and celiac ganglionectomy when carbohydrates (dextrose, lactose, sucrose) enter the upper digestive tract. Thus the participation of a depressant humoral factor (chalone?) in gastric inhibition by carbohydrates is demonstrated.

A nervous factor may normally be involved in gastric inhibition, for the normal stomach differs from the denervated tissue in the minimal quantity of carbohydrate required, in latent period and in duration of inhibition. These differences may, however, only indicate a reduced susceptibility of denervated tissue to the humoral factor.

Initiation of the humoral factor as well as the nervous factor (if present) is dependent on contact of carbohydrate with the upper small intestine for gastric inhibition follows introduction of carbohydrate into the upper intestine of a dog with obstructed pylorus or with a pouch of the entire stomach but does not result from carbohydrate in the stomach of such animals or in the auto-transplanted gastric pouch.

Autonomic reactions induced by electrical stimulation of the hypothalamus. S. W. RANSON, H. KABAT (by invitation) and H. W. MAGOUN (by invitation). Northwestern University Medical School.

Under light nembutal anesthesia the hypothalamus of the cat has been explored millimeter by millimeter with a faradic current delivered through a needle-like biopolar electrode manipulated with the aid of the Horsley-Clarke stereotaxic instrument. Sympathetic responses such as dilatation

of the pupils and a rise in blood pressure were regularly obtained from stimulation of the hypothalamus and were accompanied by an increase in rate and depth of respiration. The region giving sympathetic responses extends from just behind the optic chiasma into the mesencephalic tegmentum. Better reactions are obtained from the lateral part of the hypothalamus containing the medial forebrain bundle than from the medial part which contains the chief hypothalamic nuclei and forms the wall of the third ventricle. Stimulation of the medial forebrain bundle farther rostrally in the preoptic region fails to produce these responses.

Stimulation of the preoptic region and of the septum in front of the anterior commissure caused contraction of the bladder accompanied by a decrease in rate and depth of respiration and sometimes by a fall in blood pressure and by slowing of the pulse. No significant rise in blood pressure was ever obtained from these regions. The response from the bladder could be traced backward through the hypothalamus and stimulation of the lateral part of the hypothalamus regularly gives a contraction of the bladder along with dilatation of the pupil and rise in blood pressure. It is believed that the cell masses concerned in this reaction are situated in the neighborhood of the anterior commissure and that the pathway extends backward through the hypothalamus.

Ethyl alcohol as a fuel in muscular exercise. DAVID RAPPORT, ATTILIO CANZANELLI (by invitation) and RUTH GUILD (by invitation). Tufts College Medical School, Boston.

The gaseous exchange of a dog was obtained in a closed system, about 16 to 20 hours after a maintenance diet which was either "normal," "high carbohydrate," or "high fat." In the post-absorptive state the R. Q. of the animal at rest varied according to the previous diet, and, as in previous experiments, the R. Q. of exercise approximated that of rest, indicating the use of the same type of fuel. After alcohol, however, although the R. Q. in the resting state indicated that alcohol was being oxidized, the R. Q. of exercise reverted to that of the resting state before alcohol was administered, whether that R. Q. was high or low. The evidence obtained indicates that ethyl alcohol is not used as an oxidative fuel to perform muscular work.

Passage of native proteins through the normal gastro-intestinal wall. BRET RATNER (by invitation). Departments of Immunology and Pediatrics, New York University and Bellevue Hospital Medical College, New York.

The view most generally held to-day by the physiologist, chemist, and clinician is that undigested antigens are not absorbed through the normal gastro-intestinal wall; and that when such absorption does occur it is because abnormal or pathological conditions exist. There is some evidence for the absorption of unsplit proteins under normal conditions, but this is regarded as a fortuitous occurrence. In the light of the conflicting opinions present in the literature and the important rôle this subject plays in allergy and other conditions not yet clearly defined, we determined to reopen the problem.

The experiments were divided into 2 parts. On the one hand, guinea pigs were fed cow's milk and tested for their sensitivity either by subsequent injections or subsequent feedings. On the other hand, medical students and children were passively sensitized (Prausnitz-Küstner test)

with the serum of individuals sensitive to foods. To test their local sensitivity they were given these foods orally the following day.

The experiments on a large number of animals (493) show that at least 50 per cent can be sensitized through natural ingestion. In the human, not only with allergic, but with normal individuals, ingestion of foods, foreign and natural to the diet, may enter the circulation in demonstrable amounts.

This phenomenon is not only confined to the new-born or very young, but is also shown to occur in adults.

As no trauma to the digestive tract occurred in the course of these observations, and the experiments were carried out under generally normal conditions it is apparent that the ingested antigen must have passed the intact wall, and this passage of native protein is therefore physiological.

Pathological factors which increase permeability may undoubtedly enhance the absorption of proteins, but under normal physiological conditions this occurs with greater regularity than is generally believed.

The physiological passage of native proteins is continually impeded by certain defence mechanisms, such as hydrolysis by digestive enzymes, general impermeability of the intestines, the development of specific antibodies, urinary excretion, cooking and various methods of preparing foods, and lastly, the tendency of man and animal to eat new foods sparingly and adhere to a more or less limited diet.

The effect of corpus luteum extracts in hypophysectomized rabbits. S. R. M.

REYNOLDS and W. M. FIROR. Johns Hopkins Hospital.

Completely hypophysectomized rabbits were given five daily injections of Theelin. This treatment was followed by six daily injections of Corporin (Hisaw). Adequate operative controls were similarly treated. The difference in the endometrial response of the hypophysectomized and control animals is demonstrated.

Changes in the glomerular filtrate during passage through the tubule in amphibia. II. Urea, inorganic phosphate, uric acid and creatinine. A. N. RICHARDS, A. M. WALKER, C. L. HUDSON (by invitation) and J. P. HENDRIX (by invitation). Laboratory of Pharmacology, University of Pennsylvania.

The concentration of all these substances in the glomerular urine of frogs and necturi has been found to be the same as in plasma. Expressing the plasma concentration in each case as 1.0, the following values have been found for (I) fluid collected from the distal end of the proximal convoluted tubule and (II) fluid from the ureter.

	I	II
Urea (necturus).....	1.26 (av.)	2.1 (av.)
(frog).....	1.43 (av.)	8.0 (av.)
Inorganic phosphate (necturus).....	1.26 (av.)	2.5 (av.)
(frog).....	1.18 (av.)	2.5 (av.)
Uric acid (necturus).....	0.4 -1.6	0.55-4.2
Creatinine (necturus).....	1.13-1.9	2.0 -8.8

Reabsorption of water seems adequate to account for this relatively slight concentration which occurs in the proximal tubules. This may not apply to two experiments, not included above, in which creatinine concentration ratios were 3.0 and 4.65.

When the proximal tubule of neotoma was perfused with Locke's solution containing none of the substance in question, urea was found in the recovered fluid in concentrations up to 111 per cent of plasma, phosphate up to 71 per cent, uric acid up to 46 per cent, creatinine up to 148 per cent. When the perfusion fluid contained more urea or phosphate than the plasma, the concentration of these diminished significantly during passage through the proximal tubule. In two experiments with creatinine, it did not.

Pregnancy urine given by mouth to rats: Effect on reproductive tract and on spontaneous activity. CURT P. RICHTER. Psychobiological Laboratory, Phipps Clinic, Johns Hopkins Hospital, Baltimore, Md.

Pregnancy urine was given to twenty-eight gonadectomized male and female rats in drinking water in dilutions varying from 1:2 to 1:100. With higher concentrations the rats drank less water, but in all groups the average amount of pregnancy urine received varied from 0.25 to 2 cc. per day. Even the small doses of 0.25 cc. or less per day were sufficient to maintain normal conditions of the reproductive tract with continuous cornified smears. There was no effect in any concentration on the atrophy of secondary sex organs of males; however the great inactivity which normally follows gonadectomy was completely counteracted in males as well as females, particularly in the lower concentrations of 1:30 to 1:100 cc. per day, thus indicating that pregnancy urine contains a specific agent for activity independent of effects on sex apparatus. The reason for the greater effectiveness of pregnancy urine given this way than with extracts, may be due, either to loss of material in making extracts, or to the presence of other facilitating substances in the raw urine, possibly secretions from the pituitary. Urine from normals and castrates fails to give these effects. Amniotin in drinking water, 1:4, produces very small effects on activity and none on smears. Dried pregnancy urine given with food has powerful effects on activity and the reproductive tract. No untoward effects have been noticed in rats receiving pregnancy urine daily for as long as 80 days. This method has possibilities for clinical uses inasmuch as effective agents can be given cheaply and easily.

The reason that so much oestrin is spilled in pregnancy urine may be associated with the marked reduction in spontaneous activity which has been shown to occur at this time.

A possible explanation of the increased metabolism of diabetes. G. C. RING. Department of Physiology in the Harvard Medical School.

Observations on cats with pancreatic diabetes have shown that the metabolism remains about 40 per cent above normal, and the respiratory quotient below 0.75 unless the body weight is diminished by 30 per cent or more. With marked malnutrition caused by lack of insulin, there is a short period in which metabolism measurements show fluctuations. Then the respiratory quotient rises permanently above 0.75, the metabolism is lessened, and the acidosis disappears. I surmise that this change in respiratory quotient which follows a loss in body weight is the result of depleted fat stores and that the diminished fat combustion is very important in lessening the total metabolism. On the other hand, the augmented catabolism of diabetes would be explained by increased use of fat.

To test this explanation, I have tried, through feeding butter and cod

liver oil, to make normal and partially depancreatized cats burn large quantities of fat. In four experiments, I found a gaseous metabolism which was qualitatively and quantitatively similar to that in diabetes.

Experimental interference with conduction in the ventricle. JANE SANDS

ROBB and J. G. FRED HISS (by invitation). Laboratory of Pharmacology, College of Medicine, Syracuse University.

Experimental lesions of individual cardiac muscles have brought to light five facts related to conduction in the ventricle.

1. The elimination of each of four muscles studied produces a characteristic alteration in the electrocardiogram. Thus elimination of the Superficial Bulbo Spiral causes a slight depression of S-T in lead 1 and a slight elevation of R-T in leads 2 and 3. A similar lesion of the Superficial Sino Spiral causes a slight elevation of R-T in all leads together with a negative T. Anemia of the Deep Bulbo Spiral produces a markedly elevated R-T in all leads, while a lesion of the Deep Sino Spiral results in an elevated R-T in lead 1 and an depressed S-T in leads 2 and 3. (Robb—Proc. Soc. Exper. Biol. and Med., **31**, 311-313, 1933).

2. Subsequent experiments have shown that the two deep muscles are much more effective in maintaining circulation than are the two superficial muscles.

3. The two muscle cutting experiments described by Lewis have been repeated. It is found that mere separation of the muscle fibers longitudinally produces no change in the electrocardiogram but that when the fibers are cut transversely the electrocardiographic alteration is the same as if the blood supply had been ligated.

4. If successive branches to a given muscle are ligated at sufficient intervals the characteristic alterations of the electrocardiogram are apparent in the first record and are intensified by each additional ligation.

5. The coronary supply either to the Superficial Bulbo Spiral or to Superficial Sino Spiral *at the apex* can be ligated thus producing the characteristic change. A cut in another portion of the muscle with intact blood supply produces no further effect. This must mean that the initial lesion produced was maximal.

Aside from noting that the elimination of specific muscles produces a characteristic electrocardiogram, the associated data strongly suggest that the conduction path of the action current in the ventricle will need re-investigation.

Further studies on the effects of partial adrenalectomy on experimental diabetes. J. M. ROGOFF, B. O. BARNES, V. B. SCOTT (by invitation) and H. WARD FERRILL (by invitation). University of Chicago and Western Reserve University.

Previous studies indicated that unilateral adrenalectomy increased a dog's sensitivity to insulin and decreased the glycosuria when the pancreas was removed. These studies were undertaken to see if the loss of the medulla, the cortex, or both, were responsible for the results. The operations include denervation and partial destruction of the medulla on one or both sides, or this operation on one side with the other gland removed. The pancreas may be removed before or after the adrenal operations. Although the adrenals have been operated in several animals, we have removed the pancreas in only 3 animals thus far. In one animal the adrenal

on one side was denervated and a portion of its medulla destroyed. After removal of the pancreas, no glycosuria has occurred and the fasting (overnight) blood sugar on the fourth day after pancreatectomy was 90 mgm. The other two animals had one adrenal removed and the other one denervated with a portion of the medulla destroyed before pancreatectomy. One of these animals has shown no glycosuria and the fasting blood sugar was 92 mgm. The other animal is showing some glycosuria, but it is less than usually found after removal of the pancreas. The two animals showing no glycosuria were more sensitive to insulin than the other animal before pancreatectomy. A larger series will be necessary to see if insulin sensitivity is correlated with pancreatic glycosuria.

The metabolism of levulose. V. Influence of pregnancy. ALLAN WINTER ROWE, MARY A. McMANUS and GERTRUDE A. RILEY. Evans Memorial, Massachusetts Memorial Hospital.

Using the Hofmeister technique, i.e., graded doses of sugar with appearance of the sugar in the urine as evidence of an exceeded tolerance, the authors have studied the utilization of levulose in various phases of reproduction. Series of normal pregnant women ante- and post-partum, toxic pregnancy with and without demonstrable liver complications both before and after delivery, and post-abortion patients, have all been investigated. Pregnancy produces a general lowering of the assimilation limit toward that of the non-mature child; in the toxic cases, the superimposed effects depress the tolerance still further. Broadly speaking, however, the capacity of utilization of this sugar is somewhat less affected than is that of galactose. Further, an upward trend in the tolerance for levulose is manifest shortly after delivery in contrast to the further depression shown by galactose. The data from the series of cases of abortion reflect the duration of the pregnancy at the time of its termination. The data offer further support for the thesis that the mechanism of utilization of the two sugars is not identical although seemingly they have certain factors in common.

The biological effects of thymus extract. L. G. ROWNTREE, J. H. CLARK (by invitation) and A. M. HANSON (by invitation). Philadelphia Institute for Medical Research, the Laboratories of the Philadelphia General Hospital, Philadelphia, Pennsylvania, and the Hanson Research Laboratory, Faribault, Minnesota.

White rats (Wistar strain) have been injected intraperitoneally daily for nine months with an extract of thymus prepared by Hanson. In the first generation (F_0) treated test animals were heavier, bred more frequently, had larger litters of heavier average weight per rat; the control animals cast five litters, totalling twenty-two rats, the thymus treated pair nine litters, totalling eighty-six rats. Little of note appeared in the second generation (F_1) except somewhat more rapid development, somewhat earlier eruption of teeth and earlier descent of testes, twenty-two to twenty-nine days. This tendency becomes more pronounced with succeeding litters. In a seventh litter (L. F.) the animals were larger, body hair was visible almost from the beginning, and teeth erupted on the second day.

The third generation of thymus treated animals (F_2), as judged from forty-three surviving rats, evidenced rapidity of development almost beyond belief. Average birth weight was 5.3 grams; controls 4.4 grams (18

animals). Teeth appeared and ears opened on the first and second day. Animals were completely covered with hair and eyes opened on the fourth to sixth day. Testes descended on the twelfth and vagina opened on the twenty-third to the thirty-first day. Estrus was established from the thirtieth to fortieth day. The female of one pair (L_2F_1), injected from birth, cast a litter at forty-two days. The growth curves of third generation thymus treated rats compared with controls revealed marked acceleration with the maximum difference about the sixteenth day and approached each other after the sixtieth day. Treated animals appear unusually docile and contented.

The effects of treatment which is continued through the grandparents and parents (acceleration in the rate of development) are much more marked on the offspring (third generation) than those resulting from continuous treatment for the same length of time confined to parents alone (seventh litter first generation).

It is evident, therefore, that this thymus extract has accelerated growth, development, maturity and fertility. The effects of thymus extract become progressively more pronounced as treatment is continued into succeeding generations.

The source of the impulses underlying the effects of post-brachial transection of the spinal cord upon fore-limb reflexes. T. C. RUCH and J. W. WATTS (by invitation). Laboratory of Physiology, Yale University.

The source of the ascending impulses which underlie the augmentation of extensor reflexes (Schiff-Sherrington phenomenon) and depression of flexor reflexes of the fore limbs induced by post-brachial transection of the spinal cord has been investigated by optical isometric myographic recording of reflex contractions elicited by mechanically controlled stimulation (see THIS JOURNAL, 1933, 105, 86). The first series of experiments showed that trauma and irritation from transection is not the source of ascending impulses because both extensor exaltation and flexor depression have been repeatedly observed after post-brachial "cold-block" and "novocaine-block" of the spinal cord. The lumbar enlargement is suggested as the place of origin by experiments in which exaltation of the fore-limb stretch reflex was produced by transections in the upper lumbar region. The following observations on the rôle of the posterior roots of the lumbosacral segments in the genesis of ascending inhibition have been made. First, acute section of the 4th to 10th post-thoracic posterior roots on both sides has no effect upon the fore-limb stretch reflex. Second, bilateral section of ascending branches of posterior root fibres in the posterior columns is similarly without effect. Third, chronic section (1-14 days) proximal to the ganglion of all posterior roots on both sides below L3 fails to increase the fore-limb reflex. Fourth, augmentation of the excitability of the stretch reflex by post-brachial transection occurs even after preliminary bilateral deafferentation of all segments caudal to the transection, and the effect of transection is not detectably reduced by preliminary deafferentation. From these observations, it is concluded that the lumbo-sacral spinal segments, though completely deprived of afferent roots, nevertheless originate, or transmit from intact afferent sources, a rostrally directed stream of impulses which restrain the reflex activity of the extensors of the fore limbs. It is not decided whether the activity of deafferented segments is due to long paths circuiting through them or whether such activity signifies a spontaneous central origin of nerve impulses.

The functions of phosphocreatine in mammalian muscle. JACOB SACKS and WILMA C. SACKS (by invitation). Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor.

The amount of phosphocreatine hydrolyzed upon stimulation of the muscles of rabbits can be made to vary with respect to the amounts of lactic acid and of hexosephosphate formed. If the rabbits have been on a diet in which alkali is in excess, the amount hydrolyzed is small; if on a diet in which acidic materials are in excess, the amount hydrolyzed is markedly increased. The amount of hexosephosphate formed does not vary with this change in diet, nor does the amount of lactic acid formed.

Calculations of the amount of base liberated both by the hydrolysis of phosphocreatine and by its conversion to hexosephosphate at pH 5.6 show that in the animals which have been on an acid diet it is sufficient to buffer all the lactic acid formed. In the animals on an alkaline diet the total base liberated by these two reactions is equivalent to from $\frac{3}{4}$ to $\frac{1}{4}$ of the amount of lactic acid formed. There is probably an alkali protein complex in the muscles of the animals on a diet containing excess base. The neutralization of this probably furnishes part of the necessary buffer, as postulated by Meyerhof.

These findings are in harmony with the hypothesis that phosphocreatine has two functions in muscle. The function of the conversion to hexosephosphate is the dual one of furnishing energy for anaerobic work and of buffering against part of the lactic acid which is formed as the product of the other energy-yielding reaction in the absence of oxygen. The function of the hydrolytic reaction is solely that of buffering against lactic acid and thus maintaining a constant pH within the muscle fiber.

The effect of cholecystochinin on the resistance to the flow of bile into the duodenum. PH. SANDBLOM and W. VOEGTLIN (by invitation). Northwestern University Medical School.

The effect of cholecystochinin on the resistance to the flow of bile from the common bile duct into the intestine was carried out by using a modification of Lueth's method, which records changes in "sphincteric" or "intramural" resistance and duodenal motility simultaneously.

Twenty-five injections of cholecystochinin were made into fifteen dogs. The most constant effect was a "periodic" increase in duodenal motility (22 times). This was often combined with an increase in the intramural resistance (14 times). Twelve times this was followed by a periodic slight (2 or 3 cm. of Ringer's solution) decrease in the resistance. Only on four occasions was the intramural pressure independent of the duodenal motility, indicating a special sphincter.

It was concluded that cholecystochinin has a slight but unquestionable effect on the resistance to the flow of bile into the duodenum which in most, but not all, instances is closely correlated with concomitant changes in duodenal tone and motility.

The intrinsic regulation of the circulation of the medulla oblongata. CARL F. SCHMIDT and JOHN C. PIERSON (by invitation). Laboratory of Pharmacology, University of Pennsylvania.

The thermoelectric method of Gibbs (Proc. Soc. Exper. Biol. and Med., 1933, 31, 141), modified by the use of a negative heat gradient, was applied to the dorsal aspect of the medulla in cats. A potent vasomotor

innervation was not disclosed by stimulation of cervical sympathetic, vagodepressor, carotid sinus, or perivertebral nerves; an occasional response to sympathetic stimulation was insignificant compared with the response of the instrument, inserted in muscle, kidney, or spleen, to stimulation of known vasoconstrictor innervations; adrenalin and asphyxia produced no trace of constriction of medullary vessels. Chemical agencies, on the other hand, had constant and marked effects: asphyxia, CO_2 -excess and O_2 -lack caused increase and overventilation caused decrease in medullary blood-flow. Quantitative comparisons indicated that CO_2 has practically a specific influence, being more powerful and having a much lower threshold than changes in O_2 or pH. Apparently the intrinsic tone of medullary vessels is high, and it is opposed to varying degrees by vasodilator hormones among which CO_2 is outstanding, thus constituting an intrinsic regulation that seems to be far more important than any nervous regulation we could discover.

The effect of arsenite on medullated nerve. FRANCIS O. SCHMITT and ROYCE K. SKOW (by invitation). Department of Zoology, Washington University, St. Louis. (Read by title.)

Sodium arsenite inhibits nerve respiration and abolishes irritability in very low concentrations (order of 0.0001 M.). The effect cannot be explained on the basis of ion imbalance but involves catalytic oxidation systems. Action potential block occurs only after an induction period, the length of which varies with the season; winter nerves may not be blocked for six to eight hours while summer nerves may be blocked within two to four hours. These effects on the action potential simulate the effect of cyanide, but direct tests show that far from blocking nerve oxidase, arsenite actually accelerates this catalytic system. Arsenite does, however, block nerve dehydrogenases and the degree of inhibition shows seasonal variation, summer nerves showing a much higher degree of inhibition than winter nerves.

This opens up the possibility of determining the substrates which are specifically required for nerve conduction, the dehydrogenation of which is blocked by arsenite. Voegtlin *et al.* consider that arsenite inhibits respiration by inactivation, through compound formation, of sulphhydryl compounds in the cell. And we find that sulphhydryl in the form of cysteine or glutathione delays the arsenite block of the action potential considerably. We fail to find, however, any great protective action of sulphhydryl on the arsenite inhibition of nerve respiration.

Thermal micellar shortening of peripheral nerve. FRANCIS O. SCHMITT and LEO J. WADE (by invitation). Department of Zoology, Washington University, St. Louis.

Frog sciatics shorten when heated, the shortening beginning at about 48° and being complete by 60° . The amount of shortening averages about 40 per cent of the original length of the nerve. Claw nerves of invertebrates, as examples of nonmedullated nerves shorten as much as 65 per cent, the range lying between 60° and 90° . There are good reasons to suppose that this is due to micellar shortening of proteins of the axon, presumably the neurofibrils. Preliminary treatment with reagents such as formaldehyde, formamide, mineral acids, bases and salts, etc., which others have shown affects the reaction to heat of the protein micelles of

tendon and muscle in a characteristic manner, affects the thermal shortening of nerve in a similar manner. Moreover, heated nerve, like heated muscle, tendon, or rubber, possesses considerable elasticity, particularly if freed of the restraint of the epineural sheath.

Brodie and Halliburton who observed that thermal shortening occurs at the same temperature at which conduction fails and at which body proteins coagulate, assumed that thermal inactivation of nerve is due to coagulation of its proteins. We find consistently, however, that nerve conduction fails at a temperature from 3 to 5 degrees below that at which the first signs of shortening appear. One might argue however that this does not eliminate the possibility that the actual seat of conduction is the oriented protein micelles of the axon for the micellar rearrangements may actually begin at exactly the temperature at which irritability is abolished but that these initial changes are undetectable for mechanical reasons.

Unfortunately, no clear decision can be reached at present for we find that other major processes which must be intimately associated with nerve conduction are also considerably inhibited at approximately the same critical temperature. Thus we find that resting respiration falls to 20 per cent of normal at 45°, nerve dehydrogenases are blocked at 45°, and indophenol oxidase is blocked at 47° to 50°.

An entirely non-mechanical method for the production of automatically synchronized voltages for the spreading of the oscillograph beam and for stimulation. OTTO H. A. SCHMITT (by invitation). Department of Zoology, Washington University, Saint Louis. (Demonstration.)

In oscillographic studies on biological potentials the stimulus has, in the past, been synchronized with the sweep circuit by complex and expensive mechanical devices. The present apparatus eliminates the excessive cost of accurately machined instruments and yet retains practically all the advantages of mechanical systems and introduces a great increase in flexibility, accuracy, and range of variability. The system consists essentially of a master oscillator thyatron circuit which determines the frequency of stimulation, a second, connected, thyatron circuit which determines the velocity of sweep of the beam, and a final thyatron circuit which produces the stimulating surges. These circuits are interconnected through a vacuum tube network which permits the constants of each of the circuits to be varied independently.

This apparatus realizes complete independence and reproducibility of the following factors: 1, frequency of stimulation (range, 0.01–250/sec.); 2, velocity of spreading (range, 0.3–10,000 cm./sec.); 3, intensity of stimulation (range, 10,000 units); 4, characteristics of shock (14 variations available); 5, position of image on the screen.

Additional features are the following. Every impulse is spread regardless of frequency. The capacity between stimulating electrodes and ground is low. It is possible to spread without stimulating or to stimulate (maximum of 5,000 per sec.) without spreading. It is also possible to obtain synchronized single stimuli or burst of stimuli at arbitrary intervals. The apparatus is applicable either to direct- or to capacity-coupled amplifiers and only standard parts are used in the construction.

Thyroxin and tissue metabolism. ALEITA H. SCOTT (by invitation). Department of Physiology, Columbia University.

A study of the effect of thyroxin (Squibb's and also Harington's synthetic, crystalline compound) on tissue metabolism has been made. Blood from the alligator was the tissue studied. The method is based on gas analysis and blood gas analysis.

The oxygen consumption of the controls was 3.8 ± 0.21^1 cu. mm. per gram of fresh weight per hour at 30°C . The R. Q. was 0.87 ± 0.05 . The temperature coefficient per 10° between 25° and 30°C . was 2.02.

The oxygen consumption of the controls was 57 per cent higher during the month of June than during the winter and spring months. The R. Q. was 0.74 ± 0.07 .

The oxygen consumption of blood from animals previously injected with thyroxin was 71 per cent higher than the controls at the same time. The R. Q. was 0.76 ± 0.05 .

During June the oxygen consumption of the blood from thyroxinized animals was higher than that of the controls by 20 per cent. The R. Q. was 0.78 ± 0.07 .

Heavy doses of thyroxin (up to 15 mgm.) frequently lowered the R. Q. to below 0.70, shortly after the injection.

Thyroxin is only slowly destroyed, persistent effects being apparent six to eight months following the last injection.

The thyroxinized animals increased in weight beyond the controls. The red cells were also increased in number. There was no apparent correlation between the changes in metabolic rate and the white cell count.

Thyroxin changes the bicarbonate level and the buffering power of the blood. Thyroxin completely reverses the seasonal variations in BHCO_3 of the controls.

Thyroxin as its early effect brings about an acidosis, low BHCO_3 and pH. Later there develops a very remarkable alkalosis, high pH and BHCO_3 at the same tension of CO_2 . The buffering power of the blood from the thyroxinized animals is much higher than that of the controls during the later period.

Lactic acid formation and glucose consumption are much accelerated in blood from thyroxin injected animals.

Thyroxin added directly to the blood in some cases increased the oxygen consumption, which without a proportional increase in CO_2 formed, produces a lowering of the R. Q.

The sensitivity to insulin. V. B. SCOTT (by invitation), H. WARD FERRILL (by invitation), J. M. ROGOFF and B. O. BARNES. University of Chicago and Western Reserve University.

Using dogs as the experimental animal, a study is being made of the sensitivity to insulin in various endocrine disturbances. This report includes normal animals, hypophysectomized animals, and animals with partial adrenalectomies or denervations. The blood sugars have been followed at intervals after the injection of insulin. Both subcutaneous and intravenous injections of insulin have been made in an attempt to

¹ Deviations are mean deviations of the mean:

$$\epsilon_M = \frac{\epsilon}{\sqrt{N}}$$

where ϵ is the mean deviation and N the number of observations in the series.

standardize a procedure for insulin sensitivity. Our studies indicate that dogs with the hypophysis removed soon become more sensitive to insulin, and the convulsions which are readily relieved by glucose may recur unless large quantities of the sugar are administered. Dogs with one adrenal removed, one adrenal denervated, or one adrenal removed and the other denervated, all show an increased sensitivity to insulin. In a limited number of animals which were more sensitive than normal to insulin after the loss of one adrenal, the sensitivity increased further after the other adrenal was denervated.

Studies on pseudo-pregnancy. H. SELYE and T. McKEOWN (by invitation). McGill University.

A condition resembling pseudo-pregnancy was produced in non-lactating adult female rats by the mechanical stimulation of the nipples by an actively nursing litter.

The rôle of carbon dioxide in producing the symptoms of acute oxygen poisoning. LOUIS A. SHAW and ALBERT R. BEHNKE (by invitation). Department of Physiology, Harvard School of Public Health, Boston.

The symptoms of acute oxygen poisoning in dogs and the rôle of carbon dioxide in producing these symptoms were studied while 100 per cent oxygen at a pressure of 4 atmospheres absolute was breathed. The experiments were carried out in a compression chamber which was sufficiently large to include two operators, who were in attendance throughout the period of oxygen exposure. It was shown that the higher the alveolar carbon dioxide tension the shorter the exposure to oxygen necessary to induce the symptoms of oxygen poisoning. On the other hand, if exposure to high oxygen tensions is sufficiently prolonged the symptoms may be induced by carbon dioxide tensions that are less than normal. It is concluded, therefore, that the oxygen tension is the primary cause and the carbon dioxide tension only a contributing cause of the symptoms attending high oxygen pressures.

The circulation time of the blood in dogs before and during the digestion of food. CHARLES SHEARD, EARL C. MCCracken (by invitation) and HIRAM E. ESSEX. The Mayo Foundation and The Mayo Clinic, Rochester, Minn.

The circulation time of the blood was investigated by the use of an ensemble of physical apparatus consisting of an ionization chamber (Geiger Counter), a high voltage (to 2500 volts) power supply for the chamber, a three-stage amplifying system, and an output circuit for recording and timing discharges which occur in the ionization chamber when radiations (gamma or beta rays) are admitted to the chamber. In each experiment the injection of radium C was made into the jugular vein and the interval of time between the injection and the arrival of the radium below the ionization chamber, located over the femoral artery lying in the adductor canal, was recorded graphically. The results of the investigations show that the circulation time of the blood in dogs previously fasted is reduced 20 to 35 per cent during the digestion of a mixed meal. In one series of experiments, simultaneous records were made of blood flow (modified Rein method) and circulation time before and during digestion and it was found that increases in blood flow were coincident with the decreases in circulation time.

The total and differential white cell count in adrenalectomized rats. H. A. SHECKET (by invitation). Ohio State University.

The total and differential white cell counts of the blood of thirty-six normal albino rats were determined. These animals were then bilaterally adrenalectomized via the dorsal route at one operation. All of these thirty-six animals died of adrenal insufficiency, since other rats with adrenal "rests" were discarded.

The total white cell count, in comparison to the normal state, decreased in 53 per cent of these rats within two or three days after adrenalectomy. Before death, however, there was an increase in the total number of white cells in 89 per cent of the animals.

The differential count showed wide variations after adrenalectomy. The percentage of lymphocytes, however, was increased with a concomitant decrease in neutrophils in 52 per cent of the rats. The eosinophil, basophil, basophil and monocyte percentages showed no significant changes.

On the mechanism of action of a blood sugar raising principle extracted from the hypophysis. LEONARD B. SHPINER (by invitation) and SAMUEL SOSKIN. Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

The subcutaneous injection of 2 cc. of an alkaline extract of the hypophysis, prepared according to the method of Loeb and Bassett, was found to consistently cause a significant increase in the blood sugar level of normal dogs. While this effect varied in intensity and duration in different animals, it usually began within 15 minutes and reached its peak within two hours. Extracts of liver and muscle, prepared in the identical manner, did not show this effect.

When such a pituitary extract was injected every day or every second day, the fasting blood sugar level of the normal dogs steadily declined until hypoglycemic levels were reached. As the fasting blood sugar level decreased, the immediate hyperglycemia caused by the injection decreased or disappeared. When the administration of the extract was discontinued, the fasting blood sugar levels gradually returned to normal. Resumption of the injections again caused immediate rises in the blood sugar.

The administration of this extract to depancreatized dogs, maintained in an approximately constant diabetic state on a meat-sugar-raw pancreas diet and insulin, caused marked increases in the hyperglycemia and glycosuria of these animals. Upon repeated injection, the fasting blood sugar levels did not fall; they rose. When these animals were completely thyroidectomized, the fasting blood sugar levels and the glycosuria decreased somewhat, but the hyperglycemic action of the extract persisted.

The extract did not raise the blood sugar of ergotaminized or adrenalectomized dogs, or of animals given a high spinal anesthesia.

It is evident that the blood sugar raising principle in our extract does not act through the pancreas or thyroid gland, but through the adrenals. The pancreas is necessary, however, for the overcompensating reaction to the extract which occurs in normal animals.

Effect of adrenalectomy on chloride and water balance. HERBERT SILVETTE. Physiological Laboratory of the University of Virginia Medical School. Cats dying within two days after adrenal excision show normal or slightly

lowered serum and muscle chloride levels. Hepatic chlorides are apparently increased over the normal level. Muscle water is somewhat increased, and the liver is definitely hydrated; the water content of serum, however, remains unaltered.

Animals which survive the operation for several days (average, 7 days) show decreases in serum, liver and muscle chlorides. In these cases the blood serum is slightly dehydrated, the liver water is lower than that observed in short-surviving cats, and the water content of muscle is very considerably increased.

After adrenal removal the kidney excretes chlorides in abnormal quantities, although the fluid output is concomitantly reduced. The accompanying loss of tissue chlorides therefore appears referable to the enhanced renal excretion of the substance. With respect to water balance the conditions are reversed. The oliguria is attended by an increase of water in the tissues, and the concentration in the muscle (the main fluid depot of the body) progressively increases with the survival time.

In prolonged fasting, i.e., after the first few days, small but constant amounts of chloride are excreted in the urine. The adrenalectomized animal has a normal salt intake up to the time of terminal anorexia, but a heightened renal excretion of chlorides. In the muscle and liver of both starving and adrenaless animals the concentration of water and chlorides is approximately identical.

From this evidence it is concluded that the derangements in chloride and water metabolism observed after adrenalectomy are not specifically related to cortical function. Comparable or even more profound chloride and water shifts in the animal tissues may be produced by different experimental procedures without prejudice to normal life. In contrast, the carbohydrate deficiencies which occur in adrenalectomized animals are incompatible with life and are not nearly approximated by any other experimental condition.

A chemical consideration of red and white muscle. H. SILVETTE and S. W. BRITTON. Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

Breast (white) and leg (red) muscle and liver from 20 brown Leghorn cockerels and pullets were analyzed for water, chlorides and glycogen. White muscle contained more water and a higher percentage of chlorides than red, but only about one-third as much glycogen. The concentrations of the various constituents were found to be as follows:

Red muscle: water 77.2, chlorides 0.061, glycogen 0.24 per cent respectively. White muscle: water 76.1, chlorides 0.041, glycogen 0.89 per cent respectively. Both kinds of striated muscle thus contained less water and chlorides and more glycogen than smooth muscle from the stomach of the fowl (Evans, *Physiol. Rev.*, 1926, **6**, 358).

Some significance, as yet incompletely determined, is to be placed on the reciprocally varying water and chloride-carbohydrate relationships.

Blood sugar of the chickens averaged 157 mgm. and serum sugar 162 mgm. per cent. The glycogen content of the liver varied twenty-fold under different experimental conditions (from 0.06 to 1.2 per cent), but the hepatic water remained relatively constant at 73.3 ± 0.8 gram per 100 grams of tissue. The same lack of correlation between liver glycogen and water content, previously noticed in the mammalian liver, is therefore observed in the case of the avian organ.

Electrical activity of the motor cortex during cerebral anemia. H. N. SIMPSON and A. J. DERBYSHIRE (by invitation). Harvard Medical School.

The action potentials of the motor cortex of the cat have been studied by means of a suitable amplifier, loud speaker and cathode ray oscillograph. Under light avertin anesthesia both common carotids were exposed and ligatures placed loosely about them. The motor cortex was then exposed leaving the dura intact. The extent of the motor cortex was determined by electrical stimulation with an induction coil. Coördinated flexion of the front and hind limbs was the typical response elicited.

The spontaneous activity of this region of the cortex showed bursts of rhythmic waves of the order of two hundred micro-volts, and frequencies of 8 to 18 per second.

The large waves which appeared were similar to those described by Kornmüller for cortical fields containing large cell bodies.

Cortical anoxemia produced by traction of the ligatures on both carotids resulted in complete loss of action potentials from the motor area after approximately 20 seconds. In more than 50 per cent of the cases a definite spasm appeared characterized by hypertension of the hind limbs and clonic activity of the fore limbs, which coincided with the loss of cerebral activity. Approximately 30 seconds following the release of the carotids the cortical action potentials reappeared and simultaneously there was relaxation in the limbs.

Not all animals showed the convulsion but in those that did the cortex was always completely inactive during the period of anoxemia.

We suggest that this spasm in the cat is a release phenomenon appearing only when the cortex becomes inactive. Some of the spontaneous waves are therefore related to the cortical activity which holds in check the sub-cortical centers.

Effects of illuminating gas on reproduction in the rat. ERMA SMITH and I. WILLIAMS (by invitation). Physiology Laboratory, Iowa State College.

Female rats exposed daily for one hour in a closed circuit respiration apparatus to 1.42 per cent gas-air mixture (0.36 per cent CO) failed to rear young. The exposures were begun when the rats were 40 to 80 days of age and continued for 120 to 200 consecutive days. Ovulation occurred normally and was followed by corpus luteum formation. Occasional rats became pregnant during the early part of the experiment. In some of these resorption occurred; in others the feti died in utero; in others they were born dead or died soon after birth. No young have survived.

Male rats so treated have given rise to no pregnancies. The spermatozoa of such rats are non-motile.

There is little effect on appetite or body weight. Litter mate controls in the same room and on the same diet have given rise to numerous progeny during the course of these experiments.

The effect of a diet low in inorganic constituents on the voluntary activity of the albino rat. PAUL K. SMITH (by invitation) and ARTHUR H. SMITH. Department of Physiological Chemistry, Yale University.

Male albino rats are grown rapidly to 130 grams and then divided into three groups: I. Given *ad lib.* a synthetic diet extremely poor in ash, notably fixed base. II. Given an adequate diet in the same quantity as consumed by I. III. Given an adequate diet *ad lib.* Over a period of

ninety days the rats of group I gain only about ten grams while those of group II gain about half as much as do the rats receiving unlimited quantities of adequate food.

Voluntary activity was measured in the revolving drum-type cage. The rats of group I soon showed a distinct decrease in spontaneous activity which persisted to the end of the experimental period (90 days). This decrease in activity took place long before the running ability of the rat was impaired. In accordance with some previous observations all groups of animals tend to show a gradual decline in activity with age.

The effect of a diet low in inorganic constituents on spontaneous activity has not previously been studied. However investigations of the effects produced by complete starvation, diets devoid of protein, low in lysine content, or merely low in calories have shown that all these produce an increased activity in the rat. Anderson and Smith (*Journ. Comp. Psychol.*, 1926, **6**, 337) point out that such a difference may be due to *a*, a difference in the stage of development; *b*, a specific effect of the dietary deficiency, or *c*, a general factor conditioned by the low nutritive plane of the stunted animal, but independent of the type of stunting used.

The present experiments support the hypothesis that a change in spontaneous activity is a specific effect of the dietary deficiency.

An electron tube chronaximeter. J. M. SNODGRASS (by invitation). Oberlin College. (Demonstration.)

An electron tube device is shown by means of which the chronaxie of an excitable tissue may be measured. The measurement of time by this apparatus depends upon the charging time of a standard capacity and subsequent discharge by a Thyatron tube. The break-down of the Thyatron short circuits the electrode circuit. A substantially rectangular wave form is employed which is produced by a constant current tube. The time interval is controlled by a calibrated dial from which the reading is taken directly. The instrument permits a relatively simple calibration technique.

Method of measuring heat production in contracting muscle. C. D. SNYDER. The Johns Hopkins University. (Lantern slides.)

Recording temperature rise in contracting muscle by means of thermopile and galvanometer, the area described by the galvanometer-time curve ought to be a measure of the net heat production. Such areas measured by planimeter have already been used by the author in a study of relative amounts of heat production (*THIS JOURNAL*, in press). The present investigation is concerned with the determination of the constants necessary to be applied in order to convert unit area of galvanometer-time curves into absolute energy units. Control experiments show that the warm-junctions of thermopiles heated from 0.34 to 14.0 seconds at rates varying from 3.10^{-4} to 24.10^{-4} cal./sec. give galvanometer-time curves whose areas tend to bear a constant ratio to their corresponding maximum galvanometer excursions; the constant moreover is the reciprocal of the sp. ht. x wt. of the substance (paraffin) heated. This of itself is evidence that the relation of height of excursion to area of excursion-time curve is one of temperature rise to heat liberated. Heights of excursion and areas of galvanometer response as recorded in thermomyograms (gastrocnemius muscles stimulated with single induced shocks through their nerves) simi-

larly bear ratios to one another that tend toward constants. These latter are somewhat higher than those found in the paraffin experiments. Factors other than sp. ht. and wt. of substance, chiefly the difference in coefficients of heat-conductivity of the two substances, doubtless will account for the difference in ratio values of the galvanometer responses. The tensions developed by the muscles were varied from 370 to 1790 grams, the max. hts. of galv. deflections from 4 to 49 mm., and the areas from 63 to 775 mm.² If unit area of galvanometer deflection can be put equal to unit of heat energy a worthwhile saving of time can be gained in the analyses of the graphic record of heat production of muscle.

The hypoglycemic phase following the normal dextrose tolerance curve.

SAMUEL SOSKIN and M. DAVID ALLWEISS (by invitation). Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

In a previous communication it was shown that normal dextrose tolerance curves can be obtained in depancreatized dogs, which are receiving a constant injection of insulin plus dextrose just sufficient to maintain the blood sugar at a constant normal level. On the other hand, hepatectomized dogs receiving a constant injection of dextrose sufficient to maintain a normal blood sugar level, yield consistently "diabetic" tolerance curves. From these and correlative data, it was concluded that the pancreas is not essential to the metabolic reactions which determine the normal dextrose tolerance curve, while the liver is essential.

In two of the normal dextrose tolerance tests obtained in the above depancreatized animals, the final blood sugar values fell below the pre-test levels. The present report deals with the results of experiments designed to magnify this hypoglycemic phase and to elucidate its mechanism.

Completely depancreatized dogs were given constant injections of insulin plus dextrose sufficient to maintain a constant normal blood sugar level. After a suitable initial control period, an additional constant injection of dextrose was superimposed for three or more hours. When the injection of the extra sugar was stopped, while the original insulin plus dextrose was continued, the blood sugar fell to values well below the initial level and sometimes reached real hypoglycemic figures.

The fact that such marked hypoglycemic reactions were obtained under the conditions described, confirms the results and conclusions reported in our previous paper, and shows that the hypoglycemic phase which follows the normal dextrose tolerance curve can be explained without assuming a mobilization of insulin from the pancreas.

Studies on the heart and circulation in disease; estimations of basal cardiac output, metabolism, heart size, and blood pressure in 235 subjects.

ISAAC STARR, JR., J. S. DONAL (by invitation), A. MARGOLIES (by invitation), R. SHAW (by invitation), L. H. COLLINS (by invitation), and C. J. GAMBLE (by invitation). Laboratory of Pharmacology, and the Medical Division of the University Hospital, The University of Pennsylvania.

This study was made possible by the development of the katharometer method for the analysis of ethyl iodide by Donal and Gamble. This improvement so greatly increased the rapidity with which the cardiac output could be estimated that over 500 determinations have been made on 235 subjects. These included 31 healthy persons and 204 patients from

the wards of the University Hospital. The data contain groups of patients with intercurrent disease not affecting the circulation, with anemia, hypertension, hyperthyroidism, and neurocirculatory asthenia, also a few cases of Addison's disease, myxedema, and arteriovenous communications. Cases of various types of heart disease were studied also, e.g., patients who had once been decompensated, cases of valvular disease, and of arrhythmia, of coronary occlusion, of angina pectoris and of aneurysm. On the other hand patients with acute cardiac decompensation, with advanced pulmonary disease, or with fever, were not studied.

The results have been subjected to statistical analysis. By this means the relationship of the cardiac output to age, sex, body surface, body weight, and heart size has been studied. The normal limits of cardiac output, heart work, stroke volume and arteriovenous oxygen difference have been defined, and the deviations in disease noted. These values give information concerning the circulation, but throw little light on the condition of the heart.

The most promising method of detecting myocardial disease is by the relationship of heart work per beat to heart size, an extension of Starling's "Law of the Heart," to clinical conditions. The normal limits of this relationship have been defined: healthy persons, patients with disease not affecting the circulation, and patients with disease of the circulation but with presumably normal hearts, give values within the normal range. But in patients with undoubted myocardial disease (who had once been decompensated) this relationship is abnormal almost without exception.

Studies in the physiology of exercise. XI. The effect of physical training on the basal pulse rate. ARTHUR H. STEINHAUS and THOMAS A. JENKINS (by invitation). George Williams College Laboratory for Physiologic Research in Physical Education, Chicago.

The basal pulse rates of a number of dogs were secured under conditions prevailing for basal metabolism studies, over a period of five years. From a careful comparison of the pulse rates in these dogs taken during periods of inactivity and during periods of high physical activity leading to an excellent trained state, it is impossible to draw any definite conclusions concerning an effect of training on the basal pulse rate. In some instances the pulse rate was lowered during training, while in other cases it remained unchanged. Dogs held as inactive controls, while others were in training, at times showed drops in the pulse rate as great, if not greater, than those which were in training. In interpreting these results it is important to distinguish between *basal* pulse rate and *resting* pulse rate. The latter is the observation most commonly made by other workers.

A comparison of pituitrin, pitocin and pitressin on the water interchange in frogs. F. R. STEGGERDA (by invitation) and HIRAM E. ESSEX. Department of Physiology, University of Illinois, Urbana, and Department of Physiology, Mayo Foundation, Rochester, Minnesota. (Read by title.)

The fact that frogs injected with small doses of pituitrin, increase approximately 15 per cent of their body weight within four hours, leads one to investigate the comparative effects of pitocin and pitressin on weight changes in frogs.

Four frogs were kept almost completely submerged in water at room

temperature and were weighed accurately to 0.1 gram. Three were then injected with pitocin (0.1 cc. per 10 grams body weight); the other was kept as a control.

Following the injections, weighings were made on the four frogs at 30 minute intervals for 8 hours. The percentage weight changes in the experimental and control frogs were then calculated, averaged and plotted against time. For comparison similar experiments were done with pitressin and pituitrin.

The results indicate that pitocin increases the frog's weight about 22 per cent, whereas the frogs injected with pitressin increase only 10 per cent. Those injected with pituitrin were mid-way between with a weight increase of 15 per cent.

A comparison of the Piezo-electric myogram with the electrogram of mammalian muscle. R. H. STETSON (by invitation), H. C. STEVENS and J. M. SNODGRASS (by invitation). Psychological Laboratory, Oberlin College, Oberlin, Ohio.

The mechanical response of the gastrocnemius muscle of the decerebrate cat was recorded oscillographically by means of a quartz crystal myograph, simultaneously with the electrogram of the same muscle similarly recorded. This method of recording makes possible a rigorously isometric technique. Direct coupled amplifiers of the pushpull type were used. The system of amplification was demonstrated to be substantially without distortion by reproducing in the output a known photo-electric input. The mechanical lag in the system was shown to be substantially zero. The stimulus was a single condenser discharge graded by means of a voltage divider. The mechanical response of the muscle was measured in terms of the area of the Piezo-electric myogram by means of a planimeter. This area which represents the mechanical impulse of the muscle is the integral of $f dt$. Corresponding to the mechanical impulse is the current impulse which is the integral of idt . The quantity of current or the integral of idt is the area of the oscillographic record of the action current of the muscle. In these experiments we have compared the mechanical impulse with the current impulse as just defined, in preference to comparing maximal tension with the maximal amplitude of the action current. Our results show a definite correlation between the quantity of force and quantity of current, that is

$$\int f dt = k \int idt$$

Effects induced by the incorporation of an infusion of coffee in an adequate ration fed albino rats. PEARL P. SWANSON and CLARE A. STORVICK (by invitation). Nutrition Laboratory of the Foods and Nutrition Department, Iowa State College, Ames. (Read by title.)

An adequate synthetic diet rich in calcium and phosphorus was fed to a control group of rats; the experimental group received the same diet into which coffee had been introduced by drying an infusion on the cornstarch used in the ration. The coffee extract, prepared by vigorously boiling 170 grams of pulverized coffee and 2 liters of water for 5 minutes, was dried on 600 grams of cornstarch.

The ingestion of the coffee diet caused a decrease in the average food consumption which was reflected in a rate of growth less than that which was characteristic of the animals fed the adequate control ration. The ratio of the weight of the dried feces excreted to the weight of food consumed was greater in the rats on the coffee diet than it was in those on the adequate control diet. All of the rats were in positive calcium balance throughout the experimental period of four weeks. However, the rats fed the coffee ration excreted more calcium than did the control animals. Fecal calcium was increased by 2 per cent and urinary calcium by 19 per cent. A slight shift in the paths of excretion of calcium in the rats on the coffee diet occurred.

The calcium and phosphorus metabolism is being studied at present in rats fed the two diets in which the calcium and phosphorus have been reduced to a point just sufficient to maintain equilibrium in normal rats in order to determine whether the ingestion of coffee can induce a negative balance.

The effect of irradiated ergosterol and parathormone upon the phosphorus of the blood. J. F. SYKES (by invitation), N. B. TAYLOR and C. B. WELD (by invitation). Department of Physiology, University of Toronto.

Following the administration of parathormone to animals there is a pronounced rise in the serum calcium, but the blood phosphorus rises only slightly until the terminal upswing just before death. As the excess calcium of the serum is derived from the skeleton, one would expect the phosphorus to be mobilized with the calcium. As most previous work, however, reports only the inorganic phosphorus, it was thought of interest to investigate changes in other phosphorus fractions, and to compare the effect of parathormone and irradiated ergosterol.

Twelve dogs were used, and the average results following the administration of either parathormone or irradiated ergosterol are given in the table, where they have been correlated with changes in the serum calcium.

SERUM CALCIUM	PARATHORMONE (80-120 UNITS IN 2 DOSES)						ERGOSTEROL (0.4 CC. 10,000 × PER KG.M.)					
	Inorganic P.		Acid soluble P.		Total P.		Inorganic P.		Acid soluble P.		Total P.	
	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma
Normal	3.0	3.8	23.6	5.0	38.7	18.6	3.5	4.0	22.2	4.8	34.4	15.0
First definite rise 2-3 mgm. %	3.0	3.4	22.7	4.3	38.1	16.5	4.8	5.7	25.2	5.3	43.0	18.0
Maximum Ca = 17-22 mgm. %	3.7	4.2	24.4	4.1	39.8	17.4	6.5	6.6	24.4	5.4	46.2	20.5
Declining	5.0	5.9	26.6	7.2	49.0	19.7	6.2	8.1	26.2	7.5	47.8	22.8

It is seen that in the main the changes in the two groups are similar, except that with irradiated ergosterol the phosphorus rise is more extensive and rises with the calcium, while with parathormone the hypercalcemia precedes any change in phosphorus level. This may not indicate a fundamental difference between the actions of the hormone and the

sterol, in view of the differences in the time relationships of the curves—the action of parathormone being much more abrupt. The fact that the rise in phosphorus in no case preceded the calcium rise indicates that the primary effect of parathormone is not, as some have maintained, upon the phosphorus metabolism.

A pharyngeal inspiratory reflex of the cat. H. A. TEITELBAUM (by invitation) and F. A. RIES. Department of Physiology, School of Medicine, University of Maryland. (Read by title.)

In the cat, a pharyngeal inspiratory reflex can be elicited by tapping the infra-mandibular region just superior to the hyoid bone; or by irritating the pharynx with a cotton swab. This reflex may be absent under deep ether anesthesia. The rate and amplitude of the response vary with the degree of stimulation. Stimulation of the adjacent structures, the larynx, trachea or palate, does not elicit any inspiratory reaction. Application of a 1 per cent solution of cocaine to the pharynx abolishes the reflex. Experiments conducted on rabbits and dogs indicate that the reflex is not present in these animals.

We have been able to confirm the inhibitory effect of stimulation of the central end of the glossopharyngeal nerve of the rabbit upon respiration, as described by M. Markwald (*Zeitschr. f. Biol.*, 1887, 23, 149). In the cat, however, stimulation of the central end of the glossopharyngeal nerve increases both the rate and amplitude of respiration, with the primary effect on the inspiratory phase. Bilateral section of the glossopharyngeal nerves in the cat does not abolish the pharyngeal inspiratory reflex described above. At present, our experiments indicate that more than one afferent pathway is involved in this reflex.

The measurement of short infra-red radiation from living organisms. MARIA TELKES (by invitation). Cleveland Clinic Foundation, Cleveland, Ohio.

The aim of the present investigation has been to develop a suitable method for the detection of infra-red radiation from living organisms. With a spectrometer equipped with a rocksalt prism, vacuum thermopile and thermo-relay amplifier, the radiation emitted by the human hand was measured between 3 and 4 μ . The emission in this range was found to be identical with that of a black body at human body temperature.

For the infra-red range, shorter than 3 μ ., following the work of Coblenz, a molybdenite photo-voltaic cell has been developed which is connected with a thermo-amplifier. This device is selectively sensitive to radiations in the near infra-red field and is being used to detect the variations of radiations in this field from living organisms under varying conditions.

The effect of thyreotropic pituitary extracts on the hypophysis and the ovary.

D. L. THOMSON, H. SELYE (by invitation) and J. B. COLLIP. McGill University, Montreal.

Chronic administration of thyreotropic pituitary extracts leads to severe atrophy of the ovary in the rat. This atrophy differs from that observed after hypophysectomy by the absence of thecal deficiency cells. The hypophyses of the rats treated with such extracts frequently show degenerative changes and contain cellular elements which are similar to castration cells.

The reactions of the uterus of the rat, before and after adrenalectomy, to histamine and to anaphylaxis. CAROLINE TUM SUDEN (by invitation). Department of Physiology, Boston University School of Medicine and the Evans Memorial, Mass. Memorial Hospitals. (Read by title.)

The rat is peculiarly tolerant to histamine shock and to anaphylaxis and much more susceptible to both after removal of the adrenal glands. A study was made, in these two conditions, of the responses of the isolated and non-isolated uterus of normal rats and of adrenalectomized rats with and without autoplasmic transplants of cortical tissue. In all three types histamine, in dilutions up to 1/1,000,000, depressed the spontaneous activity of the excised uterus (46 cases). Intravenous injections of the amine, from 0.01 mgm. to 10.0 mgm. per 100 grams body weight, did not produce inhibition of the uterus in situ similar to that seen ex situ, but elicited a slight increase of tone and of frequency of contraction (36 cases). The anaphylactic response of the rat uterus in situ and ex situ was also one of mildly increased activity (120 cases). Simultaneous blood pressure determinations showed that in both operated and unoperated rats vaso-depression was the more conspicuous and consistent response to anaphylaxis (31 cases). The circulatory collapse disclosed the increased sensitivity of the adrenalectomized rats with and without cortical tissue, but the uterine reaction did not reflect this change, either in situ or ex situ.

The similarity between the responses of the uterus in situ to intravenous histamine and to anaphylaxis suggests that the evidence against the liberation of histamine or a histamine-like substance in anaphylaxis, based on the discrepancy in the responses of the excised rat uterus, may be less significant than previously supposed.

The effect of digitalis on cardiac dilatation produced by anoxemia. EDWARD J. VAN LIERE and GEORGE CRISLER. University of West Virginia, Morgantown.

The work was done on barbitalized dogs. The trachea was cannulated, a carotid cannula was inserted for blood pressure tracings and the vagi were exposed. Anoxemia was produced by allowing the animal to breathe pure nitrogen out of a bag, which was attached to the tracheal cannula. A steady flow of nitrogen was maintained. A flutter valve was arranged so that the animal expired to the outside, preventing the accumulation of carbon dioxide. The animal was kept underneath the x-ray machine during the entire experiment and a continuous blood pressure tracing was taken. A signal magnet was arranged so that the exact time and duration of the x-ray exposure could be ascertained. A time marker recorded seconds on the drum. The distance from the target to the film was kept constant at 1 meter. A 5 second x-ray exposure was used. A control x-ray picture was taken, anoxemia was then produced and x-ray pictures were taken at the end of $\frac{3}{4}$, 1, $1\frac{1}{4}$, and $1\frac{1}{2}$ minutes. The animal was allowed to return to normal.

Tincture of digitalis (0.25 cc. per kilo body weight) was given intravenously. It was allowed to act at least for an hour. A control x-ray picture was taken. Anoxemia was again produced and 4 more exposures were made at the same intervals as in the control series. The areas of the cardiac silhouettes were measured by means of a planimeter.

It was found that the heart dilated less after digitalis was administered. In some cases there was a striking difference—in others it was not so

marked, but the results all were in the same direction. In most instances digitalis caused the heart to beat slower, so changes in rate could not explain the results. Digitalis probably acted directly upon the cardiac muscle.

Carotenoids and vision. GEORGE WALD¹ (by invitation). Physiology Laboratories of the Kaiser Wilhelm-Institut, Heidelberg, and of the University of Chicago.

I have recently reported the presence of considerable quantities of vitamin A in the eye tissues of the frog and several mammals (Nature, 1933, **132**, 316). The carotenoid content of the frog eye has since been investigated in detail.

In the combined pigment and choroid layers two carotenoids are found in unusually large quantities: vitamin A ($C_{20}H_{29}OH$) and xanthophyll (lutein, $C_{40}H_{54}(OH)_2$).

In dark-adapted retinas which have been entirely cleared of the basal portions of the pigment epithelium, no xanthophyll and at most a trace of vitamin A are found. On the other hand a third carotenoid occurs here possessing properties different from any which have yet been described. I have named this substance retinene.

Retinene is characterised by the following properties: In solution in chloroform it possesses no absorption bands in the visible spectrum. It is faintly yellow, due to an ascending absorption from 500 $m\mu$ into the ultra-violet. The spectrum of the crude retinal extract possesses a small maximum at about 410 $m\mu$ and larger ones at 310 and 280 $m\mu$. The very faintly yellow solution gives with antimony trichloride a deep blue color, associated with a sharp band at 655 $m\mu$. This is at least 35 $m\mu$ longer than that of vitamin A, the nearest band produced in this reaction by any other known carotenoid.

Retinene plays a definite part in the visual process. It is found in easily measurable quantities in the retinas of dark-adapted animals. In light-adapted animals it has vanished entirely.

These facts, in addition to the recognized dependence of visual purple formation in the retina upon its contact with the pigment epithelium and upon the presence of vitamin A in the diet, suggest the following hypothesis: Vitamin A normally diffuses from reserves in the pigment-choroid into the retina. It there enters the visual purple system, from which it may be directly extracted as retinene.

Changes in the glomerular filtrate during passage through the tubule in amphibia. I. Sugar, chloride, and total molecular concentration. A. M. WALKER, A. N. RICHARDS, C. L. HUDSON (by invitation), T. FINDLEY (by invitation), and R. T. KEMPTON (by invitation). Laboratory of Pharmacology, University of Pennsylvania.

In *Necturus*, glucose in the glomerular filtrate decreases progressively as this flows through the proximal convoluted tubule. When it reaches the end, the glucose concentration is so nearly identical with that of bladder or ureteral urine as to indicate that the proximal tubule is the chief, probably the sole, site of selective glucose reabsorption.

In phlorhizinized animals glucose in fluid from the proximal tubule never

¹ National Research Fellow in Biology.

was below that of plasma. At the end of the proximal tubule glucose averaged 26 per cent higher than in plasma. When Locke's solution containing high glucose concentration was perfused through a phlorhizinized tubule, the concentration diminished significantly. It appeared that phlorhizin did not completely prevent passage of glucose from lumen of tubule into blood. When the perfusing solution contained no glucose, no more glucose entered the phlorhizinized tubule than the normal.

Chlorides are not selectively reabsorbed from the proximal convoluted tubule. Fluid collected from the distal end of this usually contains chloride in slightly higher concentration than does plasma. The distal convoluted tubule is the site of selective chloride reabsorption.

The total molecular concentration of fluid collected from the distal end of the proximal tubule is slightly less than that of plasma. In the distal convoluted tubule it decreases progressively as does the chloride concentration.

The above statements, in so far as they concern the composition of tubule fluid in normal animals, are applicable also to the frog.

The in vitro synthesis of non-fermentable reducing materials by active mammary gland. ANCEL WEINBACH and D. BAILEY CALVIN (by invitation). Department of Biochemistry, University of Missouri School of Medicine, Columbia, Missouri.

Preliminary experiments were undertaken in an attempt to demonstrate a lactase in the actively secreting mammary gland. Female rats eight days after parturition were used, since at this time the lactose secretion of rat's milk is shown to be at its peak. The glands were frozen *in situ*, dissected, frozen solid in CO₂ snow, and dried *in vacuo*.

Using serum as a substrate, with added amounts of glucose, galactose and lactose and with proper care taken to account for residual reducing material present in the prepared gland itself, it was observed that following incubation, there was an increase in the total reduction in the tubes to which gland had been added. This increase was highest where glucose was added. Furthermore, this increase was found to be in the non-fermentable fraction. Hydrolysis of this non-fermentable reducing material caused an increase in reducing power of a magnitude which would suggest the presence of a disaccharide.

Using glucose solution instead of the serum preparations, the formation of non-fermentable reducing material was again demonstrated.

An application of the Shaffer-Somogyi (Journ. Biol. Chem., 1933, 100, 695) velocity of reduction technique for sugar differentiation would indicate that this non-fermentable reducing substance might be lactose. Further confirmation is necessary, however.

Reducing power was determined by the Shaffer-Somogyi (1933) technique.

Observations on the intestinal villi and their circulation. HERBERT S. WELLS and RALPH G. JOHNSON (by invitation). Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee. (Read by title.)

A special absorption chamber has been employed which permits observation of the intestinal villi during experiments in which the direction and rate of fluid transfer through the wall of an isolated loop of gut are also

determined. No invariable correlation exists between the motor activity of the villi and the rate or direction of passage of fluid. This fact together with observations indicating that the villi do not change in volume as they shorten and that the central lacteal is not emptied by this mechanism, make it appear improbable that villus movements serve as a pumping mechanism. Isolated villi, freshly cut from the mucosa, exhibit the same type and rhythm of movement as do the intact organs. These rhythmical retractions are due to intrinsic mechanisms and would therefore seem to be myogenic rather than neurogenic in character. The retractions of intact villi are correlated with a sluggish flow of blood through the local capillaries, while a rapid flow abolishes the activity. Changes in activity seem to depend on simple chemical factors associated with processes of exchange between the smooth muscle cells of the villi and the blood.

Absorption of fluid occurs only when the villi are slender and pale and the local blood flow is intermittent and sluggish. Such an appearance suggests that the capillary pressure and hydrostatic pressure of tissue fluids of the villi are low. Secretion of fluid occurs only when the converse picture of hyperemia (active or passive, or more often both) is present. The villi are tensely swollen. Capillary and tissue fluid pressures appear to be high.

The resting gut is pale and anemic and it is an absorbing organ. Local irritation, whether mechanical or chemical or osmotic (saline cathartics or other hypertonic solutions) results in hyperemia of the mucosa, with swelling of the villi, tonic retractions that are effective in impeding the venous return, and profuse secretion. It is probable that secretion, as well as absorption, of fluid is a physical process, and that transfer of fluid through the gut wall is regulated primarily by vascular reactions, both local and general.

Effect of barbiturates on the absorption of glucose from intestinal loops. J. J.

WESTRA (by invitation) and W. A. SELLE (by invitation). Department of Physiology, University of Texas Medical School. (Read by title.)

Dogs with Thiry fistulae of the jejunum trained to lie quietly for long periods of time were used. The normal rate of absorption of glucose in a given time and from a given volume and concentration was determined previous to the administration of the barbiturate. The introduction and removal of the glucose solutions was effected by means of a catheter-balloon system which made possible quantitative recoveries. The barbiturates used were nembutal, amytal and luminal in doses of 25, 40, and 75 mgm. per kgm. respectively, injected intraperitoneally. All these substances definitely depressed the normal absorbing activity. The absorption decreased within 10 minutes after injection of the drug, reached a minimum between 30-50 minutes after injection, and then gradually returned to the normal level. There seemed to be no close correlation between the depth of the anesthesia and the depression in absorptive activity. The question of the relation between the depressed intestinal motility produced by the barbiturates and the decrease in absorption is being investigated.

Observations on the nervous control of the ileo-cecal sphincter and on intestinal movements in an unanesthetized human subject. H. L. WHITE, BETTY

MONAGHAN (by invitation) and A. S. HARRIS (by invitation). Department of Physiology, School of Medicine, Washington University. (Read by title.)

Direct observations and graphic records of intestinal movements were made on an unanesthetized human subject, a colored woman with an extensive intestinal prolapse following a colostomy at the ileo-cecal region. The observations presented here were made about 15 hours following the last meal. The proximal colon showed but little spontaneous activity; antiperistalsis was not observed. This colon is, of course, always empty. Distention of either proximal colon or distal ileum resulted in a powerful mass contraction of the former. Receptive relaxation of the proximal colon on ileac peristalsis, as described by Lyman, was occasionally but not invariably observed. The ileo-cecal sphincter was relaxed much of the time; it closed tightly on distention of the proximal colon if such distention resulted in contraction of the latter. The sphincter frequently showed rhythmic contractions, the rate being sometimes higher and sometimes lower than that of the distal ileum. Peristalsis (defined as a propulsive wave of activity resulting in expulsion of ileac contents if the wave reaches ileo-cecal orifice) occurred spontaneously several times an hour; rhythmic segmentations persisted throughout a peristalsis. The wave of contraction was not preceded by inhibition. The relaxed sphincter 1, may contract immediately on beginning of ileac peristalsis (apparently a reflex); 2, it may contract only after the wave has reached the sphincter, or 3, it may not contract at all. Peristalsis of the distal ileum frequently fails to reach the sphincter. The responses of sphincter and of ileum to adrenalin were identical; in both rhythmic activity ceased and loss of tone occurred after 0.3 to 0.6 mgm. subcutaneously. Pilocarpine, 0.07 grain subcutaneously, produced increased rhythmicity and tone of both sphincter and ileum, less marked in the former.

Filtration through cellophane membranes. H. L. WHITE, FRANK URBAN and BETTY MONAGHAN (by invitation). Departments of Physiology and Biochemistry, Washington University School of Medicine.

The relation between the filtration rates of aqueous solutions of thorium chloride through cellophane membranes and the zeta potentials was investigated. The potentials in solutions from 4×10^{-7} to 4×10^{-3} molar were determined by electrophoresis of a cellophane suspension, and the electro-osmotic transport rates across membranes in solutions from 4×10^{-6} to 4×10^{-3} molar were determined, the latter being functions of zeta. The isoelectric point with electrophoresis was at 3×10^{-6} molar and with electro-osmosis at 4×10^{-6} ; the zeta functions with the latter method are the ones concerned with the filtration results. Variations in zeta might conceivably influence filtration rate in two ways, by an electro-osmotic back-transport with stream potential as the driving voltage, and by variations in effective pore diameter due to changes in the double layer, the latter acting possibly by producing variations in the rigidity of the adsorbed water layer. If this rigidity shows a minimum at zero zeta, both factors would operate to give a maximum filtration rate at the isoelectric point. We find the maximum filtration rate at the isoelectric point, the rate being 5 per cent higher than in water. With increasing concentrations and increasing values of zeta of reversed sign the rate again falls off, being about 22 per cent lower in 4×10^{-3} molar than in 4×10^{-5} . More information on the conductivities of the membranes in the various solutions is needed in order to evaluate the rôle played by the stream potential-electro-osmosis factor.

Further study on the stimulating effect of copper on chlorophyll formation.

G. C. WICKWIRE (by invitation) and W. E. BURGE. Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

It is a recognized fact among Florida citrus fruit growers that trees set on certain types of soil, marl, for example, become chlorotic or "frenched" and that copper sulphate spread on the ground around these trees remedies this condition. Recently (Amer. Journ. Botany, 20) we determined the chlorophyll content of leaves from chlorotic orange trees before and after treatment with copper sulphate and found that this salt greatly increased the production of chlorophyll.

In the present investigation the soil on which "frenched" orange trees grew was analyzed for various constituents with a hope of determining if there was any deficiency in the essential elements that might be responsible for the "frenching." A deficiency in magnesium was found in the soil of certain parts of the grove where "frenching" occurs. It is known that magnesium is the element in chlorophyll corresponding to iron in hemoglobin and the deficiency in magnesium observed undoubtedly has entered as one cause of the chlorosis. Whether or not this is the sole cause is a question. In another part of the grove where "frenching" occurs the soil analysis showed a very low iron content. This undoubtedly has also entered as a cause of the chlorosis, since iron, although it does not enter into the constitution of the chlorophyll molecule, it is necessary for chlorophyll formation. The analysis showed further that manganese was present in very minute quantities in most of the grove land.

Analysis of the "frenched" leaves showed that they contained more iron than did the normal leaves. No manganese was found in the "frenched" leaves, but traces of this element were found in the normal leaves. So far as the mode of action of copper sulphate in stimulating the production of chlorophyll is concerned nothing seems to be known, just as nothing is known regarding the mode of action of copper in stimulating hemoglobin formation in animals. It would seem however that the two problems have much in common.

A comparison of the rate of disintegration in vitro of nucleated and non-nucleated red blood cells and the effect of copper on this rate. G. C. WICKWIRE (by invitation), L. KNEER (by invitation), H. W. NEILD (by invitation) and W. E. BURGE. Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

A few cubic centimeters of blood were drawn from the blood vessels of a man, a dog, a turtle, and a frog, placed in sterile test tubes and shaken gently while the blood clotted. A drop of blood was removed from each of the test tubes immediately and examined under the microscope. During the succeeding four days, several examinations similar to the preceding were made and it was found, contrary to our expectation, that the nucleated cells of the turtle and the frog disintegrated considerably more rapidly than did the non-nucleated cells of the man and of the dog. The nucleated cells of the turtle and frog ordinarily had completely disintegrated at ordinary room temperature after 20 or 30 hours, while the non-nucleated cells of the man and dog were apparently still in good condition. Several observations similar to the preceding were made at ordinary room temperature as well as at several different temperatures and the nucleated cells always disintegrated more rapidly than did the non-nucleated.

The addition of traces of copper sulphate to the preparations of the nucleated cells decreased markedly the rate of their disintegration. This observation suggested to us that copper might act in a similar way in nutritional anemia. That is, the copper might retard the rate of disintegration of the red blood cells in this disease and in this way maintain the blood count at a higher level. Such experiments have been begun and the effect of copper sulphate on the rate of disintegration of the red blood cells of anemic and normal rats is being studied but sufficient data have not been accumulated as yet to warrant any statement.

The chloride concentration of gastric secretion from fundic pouches and from the intact whole stomach. CHARLES M. WILHELMJ, LEO C. HENRICH (by invitation), IRWIN NEIGUS (by invitation) and FREDERICK C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska.

The chloride concentration of the secretion from fundic pouches averages between 578 and 595 mgm. per 100 cc. This value is unaltered by the rate of secretion or by the hydrogen or chloride ion concentration of the contents of the pouch. In marked contrast to the constancy found in fundic pouches, the chloride concentration of the secretion from the intact whole stomach shows marked variations, the values ranging from 340 to 590 mgm. per 100 cc.

The constancy of the chloride concentration of fundic secretion makes it possible to divide the total fluid entering the stomach into two fractions, first, the fluid of secreted hydrochloric acid which is not neutralized and second, the "extra fluid" which is composed of secreted hydrochloric acid which is neutralized, pyloric secretion and at times, regurgitated duodenal secretions. We have previously shown that the chloride concentrations of pyloric and duodenal secretions are definitely lower than that of fundic secretion, the average values being 376 and 310 mgm. per 100 cc. respectively.

Since the total fluid entering the stomach is composed of two and at times of three fluids of widely different chloride concentrations, the chloride concentration of the mixed gastric secretions will vary depending upon relative proportions of each. When little or no acid is secreted the chloride concentration of the mixed gastric secretions will be low and will approximate the value for pyloric or duodenal secretions, as more and more acid is secreted the chloride concentration of the mixed gastric secretions will rise and may approximate the value for fundic secretion, unless a large amount of duodenal fluid regurgitates into the stomach, in which case the value may be low in spite of the secretion of considerable acid.

The average composition of human duodenal secretion. CHARLES M. WILHELMJ, LEO C. HENRICH (by invitation), IRWIN NEIGUS (by invitation) and FREDERICK C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska. (Read by title.)

The duodenal contents are a mixture of succus entericus, bile and pancreatic juice in variable proportions. The frequent regurgitation of these mixed secretions into the stomach makes it desirable to know their average composition.

Forty-three determinations were made upon five normal subjects. After

passage of the duodenal tube the duodenum was stimulated by injection of tenth normal hydrochloric acid containing phenol red. In a few instances the acid solution was passed in and out of the duodenum five or six times and then removed; in the majority of instances 5 or 6 minutes were allowed to elapse after introduction of the acid, the duodenal contents were then withdrawn and placed in a sample of the same acid-phenol red mixture. Determination of the per cent of phenol red gave the amount of duodenal contents present in the sample. Total and neutral chlorides were determined after ashing. The average chloride content was found to be 272 mgm. per 100 cc. with variations ranging from 160 to 406 mgm. The average alkalinity was 0.056 normal with variations ranging from 0.02 to 0.09 normal.

An explanation of the first reflexes of cat embryos on the basis of development of reflex arcs. WILLIAM F. WINDLE (by invitation). Anatomical laboratory, Northwestern University Medical School.

The first somatic motor response to mechanical stimulation is outward movement of the forelimb in 13 to 14 mm. embryos. It occurs only while the placental circulation is intact, usually follows a perceptible latent period, often requires summation of stimuli, and appears to be followed by a long refractory period. It is executed quickly with no tetanic qualities. In these respects it differs from movements resulting from direct faradization of embryonic muscle.

All morphological elements of primitive spinal reflex arcs are present in premotile embryos. However, sensory conductors are not linked with association or motor neurones by means of sensory collaterals. These collaterals start to grow in just before the first reflexes appear. We counted them in silver stained motile and nonmotile specimens of similar sizes and ages. About two and one-half times as many collaterals occur in motile as in nonmotile ones; the greatest increase is in the brachial region. The first collaterals appear at the entrance points of dorsal roots, impinging on association neurones at the same level. The latter elements can effect connections with ramifying dendrites of motor neurones of the same segment. This is clearly a mechanism for local spinal reflexes. It is suggested that the physiological responses, so intimately coordinated with the development of the sensory collaterals, appear in consequence of their growth and are true local reflexes.

The effect of sympathetic and dorsal root stimulation on the contraction of skeletal muscle. H. G. WOLFF and McKEEN CATTELL. Department of Physiology and the Department of Medicine, Cornell University Medical College, New York City. (Read by title.)

The effect of sympathetic and dorsal root stimulation on the tension developed by the gastrocnemius of the frog has been investigated, using a strictly isometric technic with photographic recording. A series of single maximum shocks (30 per min.) was applied to the sciatic nerve and, while the response of the muscle was being recorded, the sympathetic chain (or dorsal roots) was stimulated for a short period (usually 20 sec.) by means of condenser discharges (30 per sec.). In 60 per cent of 59 animals stimulation of the abdominal sympathetic chain increased the tension of the twitch; in 20 per cent the tension was decreased; and in the remainder no change was observed. The magnitude of the augmentation averaged

4 per cent in the fresh muscle and 11.3 per cent after "fatigue" had set in. The onset of the effect began in from 2 to 4 seconds and often outlasted the duration of the stimulus. On the other hand, stimulation of the dorsal roots in 60 per cent of 35 preparations caused a decrease, but never an increase, in the tension of the twitch averaging 3 to 4 per cent. This depression in the response reached a maximum in from 2 to 6 seconds, seldom lasted more than half the period of dorsal root stimulation, and was most marked in the fresh muscle. These results may be interpreted on the basis of chemical intermediaries presumably set free in the walls of the blood vessels at the site of the nerve endings. This view is supported by the observation that epinephrin increases the tension developed in skeletal muscle and by the fact that in our experiments acetylcholine hydrobromide, perfused through the artery in sub-contraction producing amounts (0.0002 to 0.0004 mgm.), caused a depression in the tension of the twitch comparable in degree to that produced by stimulation of the dorsal roots.

The nature of autonomic effects in the submaxillary gland. H. G. WOLFF, McKEEN CATTELL and D. A. CLARK (by invitation). Department of Physiology and the Department of Medicine, Cornell University Medical College, New York City.

Comparison of the effects of stimulation of the chorda tympani and cervical sympathetic nerves on the secretion of saliva, the blood flow, and the influence of the returning blood on other organs has been made on the submaxillary gland of the cat. Stimulation of the chorda tympani is known to result in the elaboration of a chemical agent which, besides inducing salivation, has other properties resembling those of acetylcholine.¹ That chorda and sympathetic effects are essentially different was supported by the observation that secretion of saliva in a gland stimulated through its cervical sympathetic supply may be augmented by cocaine and abolished by ergotoxin without appreciable influence on the effect of chorda stimulation. On the other hand, physostigmin augments the secretion from stimulation of the chorda tympani without influencing the response to sympathetic stimulation. The differences between chorda and sympathetic effects were further demonstrated by the following experiments. The effects of sympathetic stimulation were studied by using as a test object the nictitating membrane (right) which had been previously sensitized by the removal of the corresponding superior cervical sympathetic ganglion and by the intravenous injection of cocaine. On the other side (left) branches of the cervical sympathetic distal to the point of stimulation, other than those going to the submaxillary gland, were eliminated by cutting the nerve fibers and by crushing arterial branches. Under these conditions stimulation of the cervical sympathetic nerve induced salivation, vasoconstriction (followed by vasodilatation) and, about 20 to 30 seconds later, a contraction of the denervated nictitating membrane of the opposite side, sometimes accompanied by a rise in arterial pressure. When the venous return from the gland was prevented from entering the circulation these effects were absent, although salivation was induced as before. Chorda stimulation had no such effect on the nictitating membrane. It is concluded that in the submaxillary gland of the cat, corre-

¹ Babkin, Gibbs and Wolff and Gibbs and Szelöczey.

sponding to the double innervation, salivation results through the elaboration of two chemically different agents having physiologically distinct effects.

Blood pressure and reactions to histamine in the rat after hypophysectomy and adrenalectomy. LELAND C. WYMAN and CAROLINE TUM SUDEN (by invitation). Department of Physiology, Boston University School of Medicine and the Evans Memorial, Mass. Memorial Hospitals.

There is no evidence of a hormone function for vasopressin, other than its occurrence in the posterior lobe of the pituitary. Dale (Lancet, 1929, **216**, 1179) has suggested that a steady flow of vasopressin into the circulation, rather than a fluctuating emergency output, may be a factor in the multiple provision for the maintenance of vascular tone.

Our previous work has shown that lack of the adrenal medulla alone in rats results in no lowering of blood pressure level, but produces a distinct defect in combating sudden administration of the vasodepressor drug, histamine. We now find that after posterior hypophysectomy alone the minimal lethal dose of histamine is not reduced, the blood pressure lies in the lower normal range, and the minimal dose of histamine necessary to produce a permanent fall in blood pressure is not less than that required for normal rats. After total hypophysectomy the M.L.D. of histamine may or may not be reduced to one-half, the blood pressure is below normal, but the vascular reactions to intravenous injections of histamine and of adrenalin are unaltered. The blood pressure findings parallel those of Braun Mendendez in dogs (Rev. d. Soc. Argent. Biol., 1932, **8**, 463). The changes in animals with anterior lobe deficiency reveal nothing concerning the function of vasopressin.

Elimination of two glandular factors simultaneously was accomplished by adrenalectomy together with partial or complete hypophysectomy, the rat being maintained by autoplasmic transplants of adrenal cortical tissue. This work is in progress, but with respect to blood pressure and to vascular reactions to histamine the findings are so far the same as those after hypophysectomy alone.

Our next step involves simultaneous removal of three factors: adrenal medulla, posterior pituitary, and sympathetic innervation. Until this is done no conclusions can be drawn with regard to a function of vasopressin in vascular physiology.

Vitamin G (B₂) deficiency in dogs: changes in the central nervous system, and motion pictures. H. M. ZIMMERMAN, JAMES C. FOX, JR. (by invitation) and GEORGE R. COWGILL. Departments of Pathology, Internal Medicine, and Physiological Chemistry, Yale University. (Demonstration.)

This demonstration consists in the exhibition of illustrations of histological changes induced in the spinal cord of dogs by long-continued subsistence on diets deficient in vitamin G (B₂). Motion pictures showing the behavior of such animals in the course of the experiment are also presented.

Modification of x-ray injury by carbon dioxide and ammonia. R. E. ZIRKLE (by invitation). Johnson Foundation, University of Pennsylvania.

When germinating spores of the fern *Pteris longifolia* are exposed to soft x-rays while in equilibrium at 25°C. with an atmosphere containing 20 per

cent carbon dioxide by volume, the length of irradiation required to produce a given degree of inhibition of cell division is only about three-fifths that required when the spores are irradiated in equilibrium with ordinary air. When lower or higher carbon dioxide concentrations are used, similar but smaller increases in susceptibility are observed. When spores are irradiated while in equilibrium with 0.003 N ammonia in the culture solution, the dose of x-rays required to inhibit division in a given percentage is about six-fifths that required when no ammonia is present. This decrease in susceptibility disappears when the ammonia is increased to 0.006 N, and definite increases in susceptibility occur when the ammonia concentration is raised still higher to 0.008 N or 0.01 N.

None of the concentrations of carbon dioxide or ammonia used produce any detectable effects by themselves, even when applied for longer periods than when used with the x-rays. Control experiments with nitrogen show that none of the observed modifications of x-ray injury are due to oxygen deficiency.

The modifications of susceptibility by carbon dioxide and by ammonia can be correlated upon the assumption that both agents act by changing the acidity of the protoplasm (specifically, the nucleus, in view of previous experiments). According to this view, susceptibility to x-rays in this organism is at a maximum when the acidity of the nucleus is raised to a definite degree above normal (20 per cent carbon dioxide in the gas phase) and at a minimum when the nuclear acidity is decreased to a definite degree below normal (0.003 N ammonia in the culture solution).

Spinal roots and tracts in the regulation of skin temperature. S. ZUCKERMAN (by invitation) and T. C. RUCH. Laboratory of Physiology, Yale University.

The sudden pronounced fall or rise in plantar temperature which in normal macaques follows an abrupt fall or rise in environmental temperature does not occur after transection of the spinal cord above the lumbar enlargement (maximum period of observation 3 months). The immediate post-operative state of the skin vessels appears to be that of vasodilatation and vasomotor areflexia. After a variable period vasomotor reactivity returns. After recovery, the plantar skin vessels adjust to changes in environmental temperature more slowly, less abruptly and to a lesser extent than they did preoperatively. Similar recovery occurs in the hind limb on the side of a hemisection, and if the opposite side of the cord is sectioned, the newly paralyzed limb attains an equal degree of function in less than a month.

The rare occurrence of sharp changes in plantar temperature in response to external temperature changes after division of posterior roots indicates that the normal vascular response is partly a local reflex. In a preparation deafferented in this way, the abrupt change is replaced by a slower but sometimes more extensive one, which suggests that section of posterior roots favors vasoconstriction, possibly by removal of vasodilator tonic influences.

Further evidence of tonic vasodilator action through the posterior roots is provided by the conspicuously different behavior of a hind limb retaining connection with the spinal cord only through posterior roots and that of its completely denervated fellow. The latter is usually colder than either the opposite limb or the head, and when subjected to sudden cold, falls to a

temperature even lower than that which the head reaches. Its fall approaches that shown when its blood supply is occluded. In similar circumstances, the limb with intact posterior roots (which at room temperature is usually warmer than the head) falls in temperature much less than does the denervated limb, and usually less than does normally innervated skin. The change sometimes occurs with reflex-like suddenness.

The adrenal cortex and blood sugar mobilization. R. L. ZWEMER and RUTH C. SULLIVAN (by invitation). Department of Anatomy and Department of Diseases of Children, College of Physicians and Surgeons, Columbia University, New York.

Determinations have been made of the blood sugars in thirty cats subjected to various degrees of adrenal ablation. Further studies have been made on the blood sugar changes following injections of cortin, adrenin, mixed extracts and control substances. In these additional experiments fifteen completely adrenalectomized cats, sixty-eight normal cats and fifteen rabbits were used.

The normal blood sugar of the cat averages 106 mgm. per 100 cc. of blood (96 determinations). Extirpation of the medullary portion of the adrenal seems to have no great effect (103 mgm.—23 cats), whereas the removal of one-half the cortico-adrenal tissue results in a slight diminution of blood sugar (94.5 mgm.—21 cats). Complete adrenalectomy results in a further moderate decrease during the first four days (83 mgm.—27 cats). The fifth to eleventh day average is still lower (69 mgm.—17 cats). The profound changes in blood glucose in adrenal insufficiency are essentially terminal.

Extracts of the adrenal cortex increase the blood sugar of both adrenalectomized and normal animals, but to a lesser degree and in a different manner than adrenin. Sugar reserves unaffected by adrenalectomy must be present in the body, because, with the proper stimulus, the reserve sugar can be mobilized into the blood. We do not believe that a low blood sugar is the determining factor in adrenal insufficiency, since animals may die with typical symptoms and hyperglycemia. This conclusion does not invalidate a low blood sugar as a contributory criterion of uncomplicated adrenal insufficiency. The amounts of other pertinent blood chemical constituents are, however, essential for a critical diagnosis.

The influence of the adrenal cortex on salt and water metabolism may possibly be correlated with blood sugar mobilization, since glycogen is an anhydride polymer of glucose, and the formation of glucose from glycogen requires water.

TOBACCO SMOKING IN RELATION TO BLOOD SUGAR, BLOOD LACTIC ACID AND METABOLISM

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It is a familiar observation in pharmacological experiments that the injection of nicotine may at first stimulate but later depress nerve ganglia. Its specific effect on the adrenal glands is less certain. Most observations confirm the finding of Cannon, Aub and Binger (1) that it stimulates the secretion of adrenalin. However, Kusnetzow (2) reports that perfusion of an isolated adrenal gland with a solution containing nicotine in a concentration of $\frac{1}{6 \times 10^8}$ results in significant stimulation of the secretory activity of the gland while a concentration of $\frac{1}{10^8}$ to $\frac{1}{5 \times 10^8}$ is followed first by stimulation and later by checking of secretion.

It is probable that the most effective physiological agent in tobacco is nicotine. A cigarette contains about 1 gram of tobacco and about 10 mgm. of nicotine. Lehman (3) reports that about $\frac{1}{10}$ of the nicotine is absorbed although the proportion must depend on the habits of the smoker. One might find after smoking a cigarette the blood sugar normal (too little nicotine for an effect on the adrenals) or elevated (enough nicotine absorbed to stimulate the adrenals). Conceivably after smoking several cigarettes or a cigar the blood sugar might be depressed. These suggestions, however, should not be taken too seriously because nicotine may modify the secretion of other hormones, e.g., insulin.

The fact is that all these responses have been reported. In a recent contribution Haggard and Greenberg (4) find that after smoking one cigarette the blood sugar increases 30 or 40 per cent during 15 minutes and returns to normal during the next 30 minutes. Increases of 10 to 40 per cent are reported by Lundberg and Thyselius-Lundberg (5a) after smoking a cigarette without inhaling. In their subsequent report (5b) increases in 6 experiments on 2 subjects average only 8 per cent, the maximum is reached in about 6 minutes and within 20 minutes the normal state is regained. Another type of increase is noted by Andrea (6). After smoking 2 cigarettes in 15 minutes the blood sugar rose in a fairly uniform manner over a period of two hours to a maximum about 30 per cent above the initial level. These three investigations can be harmonized if one makes the

improbable assumption that blood sugar concentration varies rhythmically after smoking.

There is one record in which consistent decreases in blood sugar were observed after smoking. Ssalischtscheff (8) on 10 subjects after smoking a cigar found at the height of the subsequent "intoxication" an average decrease of 27 per cent in blood sugar. An eleventh subject after smoking 2 cigars and a cigarette had a blood sugar of 31.

Cigarette smoking was found to be without a consistent effect on blood sugar by Burstein and Goldenberg (7). In one subject blood sugar was found to be elevated 20 minutes after smoking 5 cigarettes, and depressed 25 minutes later. A second subject after smoking 7 cigarettes first showed a decrease and then an increase. All values were within 10 per cent of the rest level. They calculate from Lehmann's data (3) that their subjects absorbed about 0.06 mgm. of the nicotine per kilo of body weight. They also studied the blood sugar in tobacco workers with an estimated daily nicotine intake of 0.1 to 0.3 mgm. per kilo. In the middle of the day 2 workers showed decreases of about 15 per cent, one an increase of 30 per cent and the fourth was unaffected.

The absence of other records indicating little or no effect of moderate smoking on the blood sugar may be due to a disinclination to publish negative results. Thus, the preliminary paper of Lundberg and Thyselius-Lundberg (5a), reporting a rise of 10 to 15 per cent within 4 minutes of starting the first cigarette of the day and as much as 40 per cent rise after the second taken half an hour later, led Dr. A. Andreen-Svedberg and one of the authors to carry out similar experiments in 1931. No significant change in blood sugar was found when two cigarettes were smoked in the course of 40 minutes. It was not planned to publish these results but the recent paper of Haggard and Greenberg (4) has led us to carry out more comprehensive experiments including observations on blood lactic acid, R.Q. and metabolic rate.

In our experiments the subjects remained at rest in the fasting state for 90 minutes before smoking and for 45 minutes afterwards. Each subject smoked one cigarette inhaling the smoke, during a period of from 5 to 10 minutes. Four makes of cigarettes were used. Capillary blood was obtained twice before smoking, one or more times during smoking and frequently during the subsequent 45 minutes. Three samples of venous blood also were obtained. Sugar was determined by the micro method of Folin and Malmros (9). It is unnecessary to present these results in detail. Nine-tenths of the 60 observations on ten subjects were within 5 per cent of the rest level without a significant trend up or down.

The report that carbohydrate metabolism is modified by smoking led us to determine the lactic acid content of blood in our experiments. A specimen of venous blood was obtained before, shortly after, and from 20

to 50 minutes after smoking. The lactic acid values, determined by the method of Friedemann, Cotonio and Schaffer (10) are summarized in table 1. Evidently in our experiments smoking has no appreciable influence on lactic acid concentration in venous blood.

In addition to observations on 10 subjects in the fasting state, similar experiments were carried out on 2 subjects beginning about 3 hours after a light breakfast. Similar results were obtained. In another experiment the subject ate a light breakfast and began smoking intensively. Within 2 hours he smoked 6 cigarettes and then had a blood sugar of 95. In the following 2 hours he smoked 2 cigarettes and a cigar. The 6 observations on blood sugar in this period ranged from 92 to 95. Smoking was then discontinued and in the succeeding 1.5 hours 5 of the 6 blood sugar values ranged from 92 to 96. An isolated high value of 120 was observed one hour after finishing the cigar. During the period subsequent to smoking hunger pangs subsided despite unchanged blood sugar values. It seems probable,

TABLE 1
Lactic acid concentration in venous blood

TIME	NUMBER OF OBSERVA- TIONS	AVERAGE	MINIMUM	MAXIMUM
		<i>mgm. per 100 cc. of blood</i>		
Before smoking.....	9	8.2	6.4	10.6
1-5 minutes after smoking.....	8	8.4	7.3	9.6
20-50 minutes after smoking.....	8	7.7	6.4	8.7

therefore, that the mechanism of hunger alleviation by smoking depends not on blood sugar level as suggested by Haggard and Greenberg (4) but on a specific inhibitory effect of nicotine on hunger contractions as has indeed been demonstrated by Carlson and Lewis (11).

It has been reported by Haggard and Greenberg that the R.Q. is increased after smoking, a change which they associated with blood sugar increase. Since our subjects showed little variation in blood sugar or lactic acid, a change in R.Q. is not to be expected. The observations presented in table 2 confirm this expectation in so far as the 8 smokers were concerned. The decrease in R.Q. in the 2 non-smokers perhaps is associated with nausea and over-breathing during the 10-minute period of smoking followed by retention of CO₂ in the subsequent periods.

Finally we may refer to the determination of oxygen consumption since this is the only function studied which showed significant alteration subsequent to smoking. It appears from table 3 that while the oxygen consumption after smoking may remain unchanged in some subjects, in others it may increase as much as 10 or 15 per cent. While this is an increase of

small magnitude, it indicates that subjects for B.M.R. determination should not smoke in the morning during which the test is to be made.

TABLE 2
Observations on R.Q.

SUBJECT	BEFORE SMOKING				AFTER SMOKING		
Smokers							
W. C.....	0.83	0.82	0.81	0.79	0.77	0.78	0.75
V. M.....	0.81	0.85	0.82	0.83	0.82	0.83	0.76
H. G.....	0.83	0.81	0.83	0.83	0.81	0.79	0.81
H. E.....	0.84	0.84	0.83	0.85	0.84	0.87	0.87
A. K.....	0.84	0.86	0.81	0.84	0.77	0.79	0.82
C. D.....	0.90	0.84	0.83	0.85	0.85	0.85	0.81
W. F.....	0.86	0.76	0.79	0.78	0.78	0.78	0.76
H. T.....	0.80	0.78	0.81	0.80	0.84	0.75	0.72
Average.....	0.84	0.82	0.82	0.82	0.81	0.81	0.79
Non-smokers							
F. C.....	0.85	0.77	0.86	0.85	0.72	0.72	0.73
S. M.....	0.82	0.80	0.79	0.85	0.74	0.70	0.79

TABLE 3
Oxygen consumption

Four consecutive periods of 12 minutes each are shown before smoking. The values in 3 similar consecutive periods after smoking are expressed relative to the mean value of the 4 control periods. The last 2 subjects are non-smokers.

SUBJECT	BEFORE SMOKING: CC. OF O ₂ USED PER MINUTE					AFTER SMOKING: RELATIVE VALUES		
	1	2	3	4	Mean	1	2	3
W. C.....	257	251	265	270	261	1.11	1.08	1.14
V. M.....	228	226	226	224	226	1.07	1.04	1.11
H. G.....	235	228	230	234	232	1.05	1.05	1.03
H. E.....	253	231	233	238	239	1.08	1.01	1.09
A. K.....	249	249	233	245	244	0.97	0.91	0.94
C. D.....	253	242	243	244	246	1.07	1.03	1.03
W. F.....	267	289	278	298	283	1.04	1.06	0.95
H. T.....	244	252	249	240	246	1.24	1.12	1.14
F. C.....	279	297	279	266	280	1.01	1.00	0.93
S. M.....	266	262	273	268	267	1.14	1.19	1.06
Mean.....	253	253	251	253	252	1.08	1.05	1.04

SUMMARY

Smoking one cigarette produced no change in blood sugar, lactic acid or R.Q. The metabolic rate was increased 5 to 15 per cent in some subjects.

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THE PRODUCTION OF THE SILENT PERIOD BY THE SYNCHRONIZATION OF DISCHARGE OF MOTOR NEURONES

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The cessation of action-currents of an extensor muscle during a tendon-jerk, which has come to be designated the "silent period," has been the object of numerous investigations designed to reveal its mechanism. The most recent theories have their origin in the study of Fulton and Pi-Suñer (1928) who pointed out that during contraction passive tension affecting the sensory endings lying in parallel with the contracted elements, e.g., the spindles, would be removed and their discharge would therefore cease. If these endings are responsible for the knee-jerk and stretch reflex, discontinuance of their afferent flow during the knee-jerk might, they pointed out, be a factor in the production of the silent period. Matthews (1931) subsequently demonstrated that such a cessation of afferent impulses does indeed occur during the motor twitch, and Bucy (1933) has presented evidence that this may be the mechanism of the lengthening reaction in man.

Somewhat earlier, Denny-Brown (1928) concluded from a careful study of the tendon-jerk that autogenetic inhibition resulting from the tension produced by the contraction also played a part in causing the lapse of action currents during the jerk. In 1920 Hoffman had suggested that the neurones taking part in the motor discharge of the tendon-jerk became exhausted of their stores of nervous energy and were therefore unable to discharge until this material was renewed. According to this hypothesis, the silent period can not be due either to an autogenetic inhibition or to cessation of afferent excitatory impulses, but to a transient depression of the excitability of the motor neurones resulting from their synchronous discharge.

Thus there are three factors that may play a part in the production of the silent period: the cessation of afferent flow during the motor twitch, the autogenetic inhibition resulting from the jerk, and the period of inexcitability in the motor neurones following their synchronous discharge.

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The first two factors might conceivably act together, as the effect of the inhibitory stimulus would be expected to be greatly increased by the interruption of afferent excitatory impulses. Recent investigations have shown that the refractory period following discharge of an extensor motor neurone may rarely exceed 6 to 10σ , but give weight to the suggestion that synchronous discharge of the motor neurones involved in the stretch might be in part responsible for the silent period.

In 1932 Eccles and H. E. Hoff published the results of a study of the rhythmic discharge of single motor neurones in de-afferented preparations which showed that the premature discharge of a neurone caused by an antidromic volley was followed by a pause as long as or longer than the normal interval between successive discharges, which varied from 43 to 155σ . The possibility therefore exists that the efferent volley of the jerk synchronously discharges all the active neurones which have then to remain quiescent for a time equal to or slightly longer than that between their normal successive discharges. An experimental reinvestigation of the silent period to evaluate the rôle of the three possible mechanisms: 1, autogenetic inhibition; 2, subsidence of afferent flow, and 3, synchronous re-setting of the efferent units, was therefore considered advisable.

METHOD. These studies have been made on cats decerebrated by the trephine method under deep ether anesthesia. In some experiments the quadriceps muscle was used, while in others soleus or gastrocnemius was prepared. All muscles but the one to be used were immobilized by appropriate nerve or tendon section, and the limb rigidly attached to the heavy Sherrington table by drills in the femur or tibia. Stimulation was by means of silver-silver chloride glass shielded electrodes which permitted the nerve to remain in situ.

Records of action currents were obtained by means of silver-silver chloride pins inserted in the muscle, one in the belly of the muscle and one near the tendon, leading to a string galvanometer. Myographic tracings were taken with a Sherrington isometric shadow myograph arranged in the optical system of the galvanometer to permit simultaneous recording of both action currents and tension.

RESULTS. A. *The silent period with extrinsic excitation.* If the silent period is due only to a subsidence of afferent excitatory impulses during the jerk, this action would be expected to be confined to those neurones which are discharging in response to the stimulus of the stretch. Other neurones, caused to discharge by means of a constant flow from some other source should not be affected by the cessation of discharge from the stretch afferents, and should show no silent period. It is true that some of these neurones may be facilitated by the stretch reflex. When this facilitation is removed by cessation of the afferent flow during the jerk, there might be insufficient excitation from the external source to permit discharge and

a silent period might be produced in a few neurones. In all but the weakest reflexes, however, this factor would play but a small part. Accordingly, tendon taps were elicited in both quadriceps and soleus during various combinations of stretch reflex and crossed extensor reflex.

In all but the very strongest crossed-extensor reflexes, silent periods were obtained in the entire pool of discharging motor neurones. With very light crossed-extensor stimulation the silent periods obtained were almost equal to those without additional extrinsic stimulation, but as the crossed-extensor reflex increased in intensity, the silent period shortened, and with such strengths of crossed extensor reflexes that the tendon tap no longer produced distinguishable action currents, the silent period was absent. Thus, in one experiment, three successive tendon taps during the course of a stretch reflex alone induced silent periods of 150, 170, and 170 σ . After a very weak crossed-extensor reflex was elicited in addition to the stretch reflex, two taps were followed by silent periods of 135 σ . After further recruitment by increasing the intensity of crossed-extensor stimulation, silent periods of 45 to 75 σ were obtained. When the crossed-stimulation was stopped after a tendon-jerk with a silent period of 75 σ , the next silent period lasted 130 σ . In the last records on the same plate, the crossed-extensor reflex was strengthened, so that the action current of the taps could hardly be identified, and following these taps there was no silent period. In another experiment silent periods following tendon taps with a stretch reflex alone lasted 80 to 95 σ , while with crossed-extensor stimulation the silent periods were reduced from 40 to 55 σ , and in a third experiment the silent periods with stretch only were 70 to 80 σ in duration and with an additional crossed-extensor reflex lasted only from 45 to 50 σ .

The tendon tap was therefore followed by silent periods in units which the stretch reflex was not activating and which could not consequently be caused to stop discharging by the cessation of the discharge from the stretch afferents. There must therefore exist some additional mechanism or mechanisms which are capable of producing a silent period.

The shortening of the silent period with additional extrinsic excitation is very similar to, if not identical with, the shortening in the silent period noticed with increasing autogenetic excitation produced by augmenting the stretch. In one experiment with a stretch reflex so weak that individual units could be identified, the silent period after tendon taps varied from 95 to 114 σ . With increasing stretch such that individual units could no longer be identified, silent periods ranging from 90 to 95 σ were obtained, and with a still greater stretch, silent periods of 80 to 95 σ were found. In another experiment silent periods of over 200 σ followed taps in a light stretch reflex, and with increasing stretch the silent period gradually decreased to 70 σ , with a moderately intense background. The decreasing silent period with increasing crossed-extensor background does not there-

fore indicate necessarily that these silent periods were produced by a mechanism different from that which caused the silent period in the stretch reflex.

These experiments give no evidence that the cessation of afferent flow does not play a part in causing the silent period in the neurones responding to the stretch. They do, however, indicate that another mechanism exists which can give rise to silent periods in the rhythmic discharge of neurones other than those taking part in the stretch reflex.

B. The silent period in other muscles. In 1928 Denny-Brown concluded that the silent period was the result of central inhibition because he found it in muscles other than the one responding to the tendon tap and published a figure in which the silent period seemed to be present without a preceding efferent volley.

In two of our experiments silent periods were obtained in the soleus muscle when the tendon of quadriceps was tapped. In both instances silent periods were produced in the action currents of stretch reflexes of the soleus muscle of such vigor that there existed no normal gaps in the electrical record of a length that might be confused with silent periods. In both of these experiments the silent periods were always preceded by a synchronous efferent discharge of considerable magnitude. That the phenomenon in soleus was not caused by some accidental stretch to soleus was demonstrated by the persistence of the jerk and the silent period even after all connection between the lower and upper parts of the limb had been separated with the exception of the nervous and vascular supply.

It has therefore been impossible to confirm Denny-Brown's observation that a tendon-jerk in one muscle can be followed by a silent period in the discharge of another extensor muscle without a preceding motor discharge.

It must be concluded that the muscular jerk in the soleus muscle and its related action current were produced by a spread of the afferent impulses, arising from the tap on the tendon of the quadriceps, to the centers of the soleus muscle. The silent period in the action currents of the stretch reflex in the soleus muscle may, therefore, have the same explanation as the silent period in the electrical manifestations of the quadriceps muscle, both being secondary to the preceding jerk and synchronized efferent impulses.

C. The silent period with reduced efferent outflow. If the silent period is caused by inhibition set up by stimulation of sense organs in the muscle or tendon as a result of the contraction brought about by the tendon tap, reduction of the size of the efferent discharge would be expected to decrease the intensity of autogenetic inhibition and therefore reduce the length of the silent period. Accordingly in two experiments the efferent roots supplying the muscle (in this instance the soleus) were progressively cut down and the silent periods at each stage were compared. No changes whatever

were noticed, except that in one experiment in the final stage only one unit remained, and the silent period obtained in it was longer than that obtained previously.

The length of the silent period is thus unaffected by the number of units taking part in the jerk as long as they include all the units participating in the stretch reflex. The inhibition arising from the stimulation of muscle sense organs by the contraction of the jerk, if it exists at all, must therefore play a minor part in the production of the silent period.

D. *The silent period and the pause following an antidromic volley.* Eccles and H. E. Hoff (1932) were able to demonstrate that when the axon of a rhythmically discharging motor neurone is stimulated by a single break shock, an antidromic impulse is conducted to the motor neurone via the motor fiber, and "resets" its rhythm in such a way that a pause ensues that is equal to or somewhat longer than a normal rhythmic interval. In a similar way a premature discharge induced in the pacemaker "resets" the rhythm of the heart (Eccles and H. E. Hoff, 1931). If several motor neurones are discharging, an antidromic volley synchronizes the discharging units and is followed by a pause limited in length by the rate of the most rapidly discharging neurone.

To compare the length of such a "silent period" with the silent period of the tendon tap, in four experiments the right soleus muscle was de-afferented by section of the right 6th, 7th and 8th lumbar roots, which has been found to sever completely the afferent connection of the popliteal nerve. A crossed-extensor reflex was elicited in soleus by faradic stimulation of the contralateral sciatic nerve and in the course of this reflex the nerve to soleus was subjected to a single break shock. In three further experiments this procedure was carried out in preparations in which the silent period of a tendon-jerk in soleus had already been studied. Pauses thus produced in the activity of a group of discharging soleus motor neurones were entirely comparable to the silent periods produced in soleus action currents by means of a tendon tap.

Figure 1 shows silent periods of tendon taps in soleus compared with "silent periods" produced in de-afferented soleus muscles by an antidromic volley set up by a single break shock applied to the motor nerve. In length and in the complete absence of action currents, the silent periods of these two different origins are strikingly similar. Thus, in the experiment of November 28, 1933, a tendon tap produced silent periods in soleus varying from 70 to 220 σ , varying inversely with the intensity of the background induced by the stretch reflex. The animal was then lightly anesthetised with ether and the muscle de-afferented. After recovery from the anesthesia a crossed-extensor reflex was elicited and single antidromic impulses sent up the motor nerve fibers. By this procedure "silent periods" of from 60 to 190 σ were produced, depending upon and varying inversely with the intensity of the crossed-extensor background.

When a similar experiment was tried with quadriceps results were the same. The stretch reflex in quadriceps can bring into activity only a

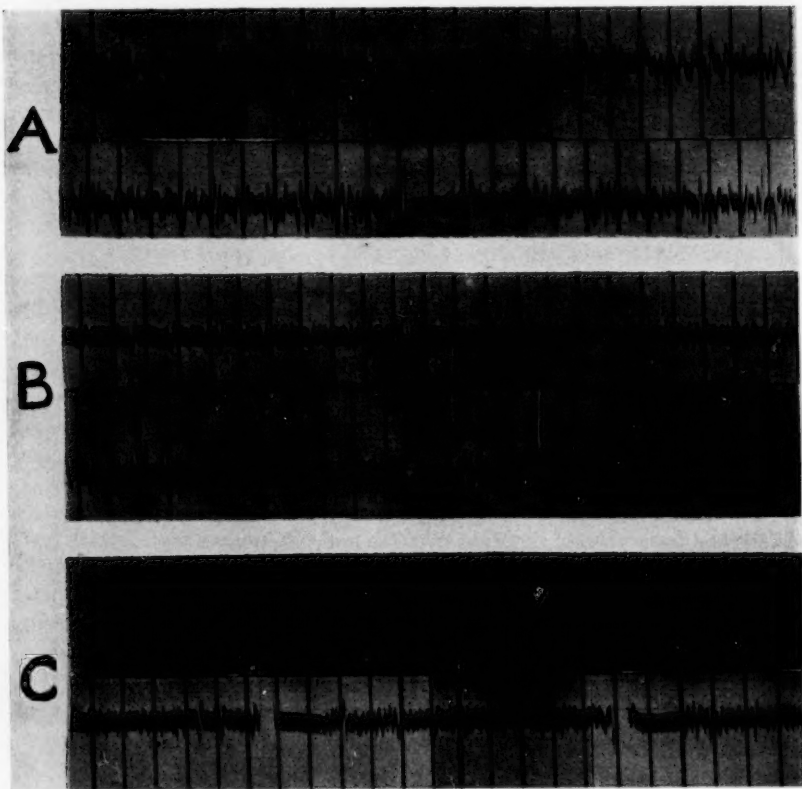


Fig. 1. Experiment March 10, 1934. Decerebrate cat. Records were obtained first of tendon jerks in the right soleus against a background of stretch reflex. The animal was then lightly anesthetized with ether and the 5th, 6th, 7th and 8th lumbar dorsal roots on the right side were severed. After recovery from the anesthetic shielded electrodes were placed on the right popliteal nerve and single break shocks delivered during a crossed-extensor reflex elicited by stimulation of the left popliteal nerve. String galvanometer records of the muscle action currents were taken.

A. Above is a record of the silent period of a tendon jerk lasting 66σ from the beginning of the active current of the jerk. Below is the silent period following a single antidromic volley. It has also a duration of 66σ .

B. Above the silent period of a tendon jerk and below the silent period following an antidromic volley, both have a duration of 60σ .

C. Two silent periods following tendon jerks and two silent periods following antidromic volleys. All show complete absence of action currents for 80σ .

small proportion of the total motor neurone pool, so it was difficult to obtain, by means of crossed-extensor stimulation, a discharge of only those units which the stretch reflex activates. When, however, an electrical, record of the action currents was obtained, with the crossed-extensor reflex, comparable to that produced by the stretch reflex the silent period following an antidromic volley in the motor fibers of quadriceps was identical to that following the knee-jerk. With more intense crossed-extensor stimulation, however, the rate of the discharging neurones increased greatly and the length of the "silent period" of the antidromic volley decreased, just as the length of the silent period following the knee-jerk had been previously observed to decrease with increasing intensity of the stretch-reflex.

It was therefore possible, by a procedure which caused synchronous discharge of the active motor neurones in which neither cessation of afferent flow nor inhibition were involved, to produce a temporary cessation of action currents comparable in every way to the silent period following a tendon-jerk.

E. The silent period in the discharge of single units. In some instances, especially in soleus, where the discharge of single units may more easily be recorded, it was possible to obtain records of jerks elicited by tendon taps during the rhythmic discharge of single motor units. In these experiments the tendon was not tapped directly, to avoid harmful stimuli (see fig. 3) to the tendon but the reflex was elicited by tapping the foot and thus stretching the muscle. Taps were strong enough to give rise to vigorous responses. Records of these experiments showed that the efferent volley had an action similar to that of an antidromic volley (Eccles and H. E. Hoff, 1932) or of an extrasystole in the pacemaker of the heart (Eccles and H. E. Hoff, 1931) in causing a "resetting" of the rhythm of the motor neurone so that an interval equal to or slightly exceeding a normal interval existed between the jerk and the next discharge. The length of the interval or "silent period" was shown to depend only upon the rate of discharge of the single unit.

On November 10, 1933 a unit was obtained in one record which beat very regularly at an interval of 115σ , and a tendon jerk occurring 75σ after one discharge was followed by another discharge after 115σ . A tendon tap in another rhythm which varied from 130 to 150σ was followed by a silent period of 140σ . In other records in the same experiment the silent periods were almost exactly equal to the rhythmic interval, while in others they were slightly longer (see fig. 2). The data at present available are insufficient to indicate whether there is any relationship between the position of the jerk in the cycle and the subsequent silent period.

Even in those cases where the silent period following the jerk was slightly longer than the normal rhythmic interval, the next interval was not prolonged and showed only a normal variation in length.

In no record could any sign of inhibition be demonstrated. In the paper of Eccles and Hoff it can be seen that the normal interval may vary fairly widely. Further, a jerk early in the cycle might possibly be followed by a period of about 1.5 times the length of a normal interval and allowing for

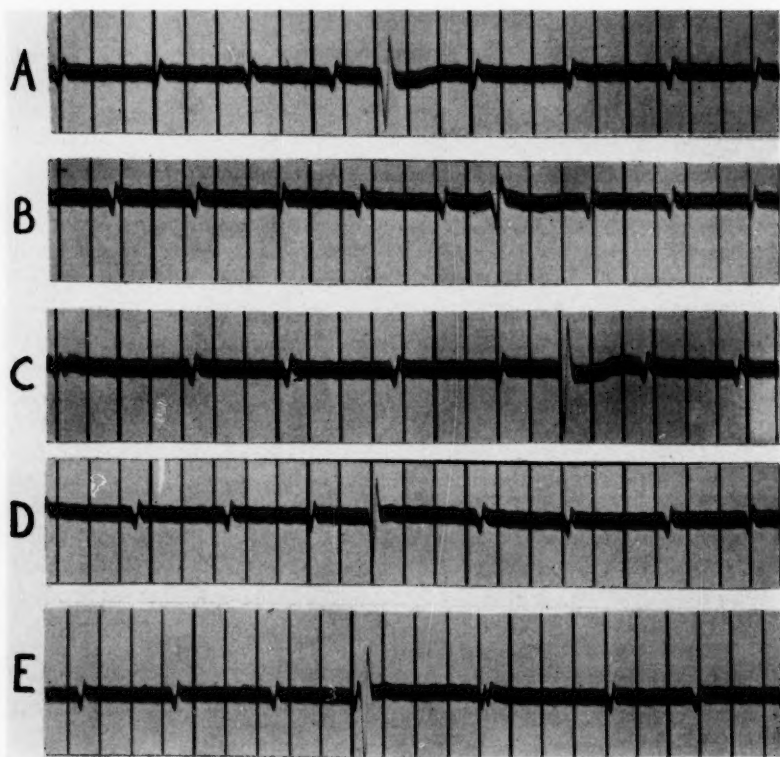


Fig. 2. Experiment March 20, 1934. Decerebrate cat. Single motor unit of right soleus isolated because of its low threshold and its location near the edge of the muscle. Rate about 8 discharges a second. In A the interval following the tendon tap, seen as the large action current in the middle of the record, is almost exactly equal to the average rhythmic interval.

In B the interval following the tap is slightly longer than the average, and in C, somewhat shorter. In D the interval following the tap is 20σ longer than the average interval, and in E, the very early jerk is followed by an interval 1.3 times the average interval.

individual variations, as long as 1.7 times a normal interval. Intervals longer than this have not been found.

The conditions of these experiments were especially favorable to the demonstration of any inhibition that might be present. A light background of stretch reflex and a very slow rate of discharge, pitted against a brisk tendon tap would tend to magnify greatly the effects of central inhibition. Despite these favorable circumstances no signs of inhibition could be detected.

These experiments indicate that the major factor in the genesis of the silent period is the resetting of the rhythm of the active neurones by their synchronous discharge by the efferent volley of the tendon jerk. With light reflex background the rate of the discharging units is slower and the silent period will, therefore, be longer. With more intense background

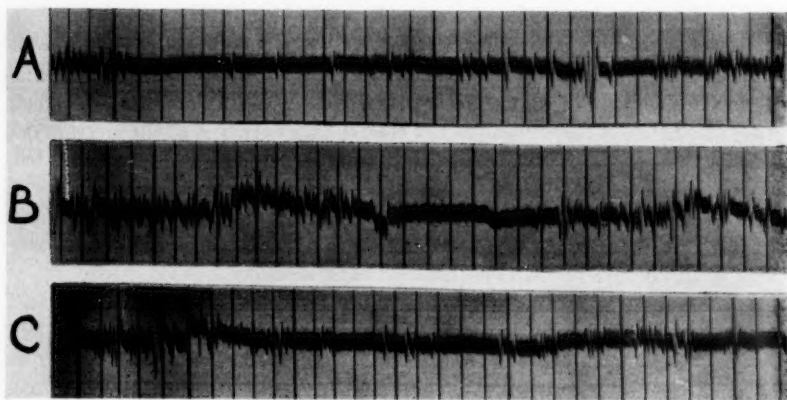


Fig. 3. A, B and C. Decerebrate cat. Soleus muscle. Asynchronous discharge of a number of units responding to stretching the muscle. Each record shows inhibition of discharge caused by pressure with the handle of a scalpel on the tendon near its insertion. Very strong tendon taps would probably involve the end organs responsible for the inhibition shown here.

the rate of the discharging units increases, and new units of higher thresholds and more rapid rate of discharge are recruited, and the silent periods are therefore shorter.

The similarity between the silent period of the tendon jerk and the pause following an antidromic volley in the motor fibers can therefore be understood. Both have as their basis the synchronous discharge of all active neurones, in one case by the efferent volley of the jerk, and in the other by the antidromic volley. In both cases the rhythm of the discharging neurones is reset, and a pause occurs equal in length to the interval between successive discharges of the most rapidly firing motor neurones. That such is the case is further supported by the fact that the length of the silent period

is always within the magnitude of the rhythmic interval occurring in the discharge of the motor units, whereas if inhibition were a factor it might be anticipated that with the more intense muscular responses silent periods of greater length would be found.

DISCUSSION. Premature discharge during the rhythmic activity of the motor neurone has been known to reset the rhythm of the neurone and produce a pause equal to or slightly longer than a normal rhythmic interval. This occurs not only with early discharges caused by an antidromic impulse but also follows early beats of intrinsic origin. Such behaviour seems to be a general property of rhythmic mechanisms, and has been also demonstrated in the pacemaker of the mammalian heart by Eccles and H. E. Hoff (1931) and in the rhythmically discharging sense organ by Matthews (1931).

These experiments demonstrate that a tendon tap evokes a synchronous, and in the case of many of the units premature, efferent discharge which resets the rhythm of all the active neurones in a similar manner. With only one neurone responding to the stretch this resetting of the rhythm can be clearly shown. When more than a very few units are discharging their synchronous activity provides a continuous background of action currents so that when the rhythm of each unit is reset by the synchronous discharge of the jerk, a silent period is produced equal to the interval between successive discharges of the most rapidly firing neurone.

CONCLUSIONS

1. All phenomena associated with the silent period are accounted for on the basis of the "resetting" of the rhythmic discharge of the neurones taking part in the stretch reflex by their synchronous discharge in the tendon jerk.
2. The subsidence of afferent flow resulting from the release of tension of "in parallel" sense organs is not the chief mechanism for production of the silent period of the tendon tap.
3. Neither autogenous inhibition from the tendon jerk, nor direct inhibition from the tap itself can be demonstrated as the cause of the "silent period" following the knee-jerk.

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THE MECHANISM OF GASTRIC MOTOR INHIBITION FROM INGESTED CARBOHYDRATES

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The regulation of gastro-intestinal motility has been ascribed predominantly to nervous mechanisms. Actually, motility, like secretory activity of the digestive tract, may be controlled by nervous and also *humoral* factors. Experiments demonstrating the regulation of motor activity by naturally occurring blood-borne factors are therefore particularly significant.

Humoral factors are involved in the inhibition of gastric motility which follows the ingestion of fats (Quigley, Zettelman and Ivy, 1934) and the similar action of carbohydrates on the stomach suggests a dependence on the same general mechanism. The vagi are not essential in the latter phenomenon, for Templeton and Quigley (1930) observed inhibition of motility in a Heidenhain pouch and Quigley and Hallaran (1932) in the vagotomized stomach following ingestion of carbohydrates. Gastric inhibition from carbohydrates depends on their presence in the digestive tract but not on the presence of carbohydrates *per se* in the blood stream, for inhibition does not follow the intravenous injection of dextrose or lactose (Quigley and Hallaran (1932), Mulinos (1933)).

The present investigation was designed to determine the effect of ingested carbohydrates on the denervated stomach and also whether the gastric inhibition was dependent on carbohydrates limited in contact to the stomach or to the intestinal mucosa.

EXPERIMENTAL. The animals employed throughout this investigation were prepared by surgical methods previously described (Quigley, Zettelman and Ivy) appropriate for the individual phases of the study and subsequently were maintained in a healthy condition. They were trained to lie fully relaxed on comfortable pads during each experiment at the beginning of which they had been fasting for twenty-four hours. Gastric motility was studied by the balloon method and a tube which passed through and for a short distance beyond the balloon was introduced into the stomach or pouch with the balloon to be used for injecting solutions (at body temperature) while motility was being recorded.

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A. Inhibition of gastric motility in the denervated stomach. 1. Autotransplanted, denervated pouches were prepared in five dogs and simultaneous records of motility were made from the pouch and the remaining portion of the stomach. Introduction of carbohydrate solution into the stomach in 89 experiments (35 dextrose, 13 lactose, 41 sucrose) gave results from the stomach of these animals identical with the normal stomach as described by Quigley and Hallaran. The administration of 100 cc. 6 per cent dextrose during a 5 minute interval usually produced complete inhibition within 3 minutes (time intervals in all cases are measured from the *beginning* of the injection) and recovery began in 15 to 45 minutes; 11 per cent lactose or sucrose solutions acted similarly. The results were more striking, more conclusive and more constant in occurrence when solutions of higher concentrations were employed and dextrose, lactose and sucrose as 20, 30, 40 and 50 per cent solutions, usually in amounts of 100 cc., were used in the majority of the experiments.

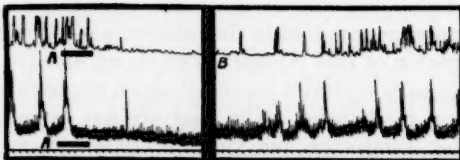


Fig. 1. Simultaneous records of spontaneous motor activity from the autotransplanted pouch (above) and the stomach (center). Lower record 1 minute time intervals. Horizontal bands A indicate period of injecting 100 cc. 11 per cent sucrose into the stomach; B, 24 minutes after beginning of sucrose injection shows recovery of pouch and stomach.

The response of the denervated pouch to such administrations of carbohydrate closely paralleled that of the stomach in all respects (fig. 1), but inhibition of the pouch was later and more gradual in onset, developing in 4 to 5 minutes and recovery usually occurred first in the pouch, thus the period of pouch inhibition was slightly the shorter.

Both the stomach and pouch usually showed a decrease in motility (frequency and amplitude of contractions) and in tone. Motility and tone were usually modified in the same general direction but not necessarily simultaneously or identically; complete disappearance of motility might occur without significant change in tone and minimal tone frequently developed several minutes after complete inhibition of motility. Recovery of tone was a more gradual process than return of motility and in the pouch return of normal amplitude of contraction usually preceded recovery of frequency.

Carbohydrate solutions of various concentrations displayed quantitative

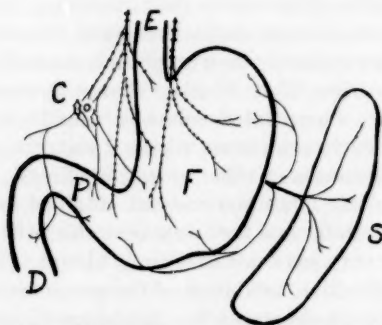
rather than qualitative differences in their inhibitory action. With weaker solutions the onset might be moderately delayed, frequency of contractions was depressed more completely than amplitude, especially in the pouch, and recovery occurred earlier (15 to 35 min.) than with concentrated solutions (2 to 3 hrs.). The concentrated solutions usually produced abrupt inhibition of the stomach, sometimes within 2 minutes, but gave no indication of producing nausea or other vitating effects.

Typical carbohydrate inhibition was not obtained in every experiment. Good preinjection motility and tone were desirable; with low grade activity inhibition might be very moderate or entirely absent. If the stomach were quiet and the pouch active, inhibition of the pouch usually occurred without appreciable effect on the stomach. Inhibition of the pouch or stomach failed to occur in a few experiments following the administration of an amount of carbohydrate shown at other times to be effective. The failure might be due to a slow entrance of carbohydrate into the duodenum, (the upper intestine will subsequently be shown to be the site of initiation of the inhibition), thus indicating a dependence upon a time-quantity factor. On rare occasions typical inhibition of the stomach occurred with little or no effect on vigorous pouch activity. This was explained by assuming a lowered susceptibility of the pouch to the humoral inhibitory factor or by assuming that the stomach is inhibited through nervous pathways in addition to the humoral mechanism. Other observations supporting these conclusions relate to the earlier onset of inhibition and its longer duration in the stomach.

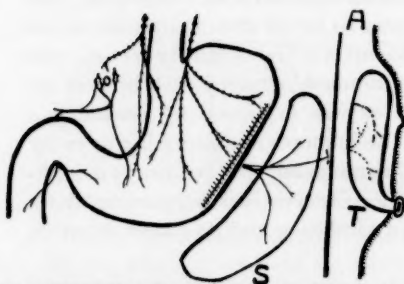
Inhibition of the pouch and stomach by dextrose, lactose and sucrose was essentially the same, but dextrose was more effective in degree and duration of the inhibition. The effectiveness of carbohydrate inhibition is exemplified by the fact that 100 cc. 50 per cent sucrose completely inhibited the marked hypermotility of pouch and stomach produced by 4 mgm. of pilocarpine.

Gastric inhibition from the carbohydrate solutions was not due to their fluid content, for 100 cc. of 0.9 per cent NaCl may be introduced into the stomach without producing depression (Quigley and Hallaran). We have fully confirmed this observation and extended it to show that the pouch also is not affected. We have likewise confirmed and extended their observation that hypermotility of gastric tissue does not follow the recovery from carbohydrate inhibition. The motility of pouch and stomach which occurs after recovery can be again inhibited in the characteristic manner by repeating the administration of carbohydrate.

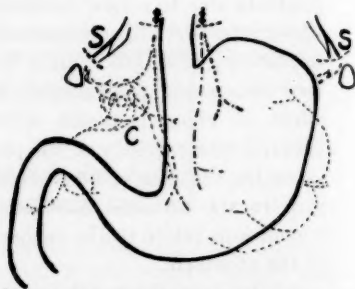
2. The extrinsic gastric nerves were sectioned in one dog by performing double vagotomy, double splanchnicotomy and celiac ganglionectomy. The action of carbohydrates introduced into this stomach was studied in nine experiments (5 dextrose, 4 sucrose) by the method previously de-



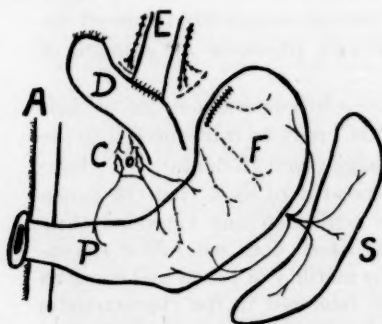
NORMAL STOMACH-Normal Innervation, E. Esophagus
F. Fundus, P. Pylorus, D. Duodenum, C. Celiac Ganglion
S. Spleen.



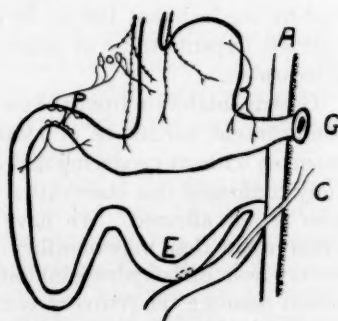
AUTO-TRANSPLANTED POUCH-All extrinsic nerves and short paths to pouch cut, T. Transplanted pouch, A. Abdominal wall, S. Spleen.



VAGOTOMIZED, SPLANCHNICOTOMIZED and CELIAC GANGLION-ECTOMIZED STOMACH-All extrinsic nerves sectioned, short paths intact, S. Splanchnic nerves, C. Celiac ganglion.



ENTIRE STOMACH POUCH-Vagi and short paths to esophagus and duodenum cut, sympathetic fibers intact, E. Esophagus, D. Duodenum, C. Celiac ganglion, F. Fundus, P. Pyloric antrum, A. Abdominal wall, S. Spleen.



PYLORIC OBSTRUCTED STOMACH-Normal innervation, G. Gastrostomy opening, C. Catheter into jejunum for feeding, E. Enterostomy opening, P. Mucosal suture obstructing pylorus, A. Abdominal wall.

scribed. Results almost identical with those from the normal stomach were obtained, but during the injection period in about half the experiments an elevated base line and an increase in amplitude and frequency of contractions developed. This was rather definitely a stretch phenomenon related to the increased pressure within the stomach. Complete inhibition occurred slightly later (4 to 5 min.) even with concentrated solutions and was more gradual in development than in the normal stomach.

B. Site of initiation of the inhibitory factor. While recording gastric motility, carbohydrate solutions were introduced into the stomach under conditions which precluded escape into the intestine. Concentrated dextrose solutions injected into the pouch of the entire stomach (3 experiments) or into the stomach of a dog having a mucosal septum at the pylorus (2 experiments) did not inhibit gastric motility (occasionally there was a slight increase in motility and elevation of the base line). In 15 experiments dextrose or sucrose solutions (40 to 70 per cent) were introduced into the auto-transplanted pouch without altering motility in the main stomach on any occasion. In some experiments the pouch record showed a marked and abrupt elevation of the base line usually accompanied by an increase in rate but a decrease in amplitude of contractions (incomplete tetanus). This resulted from increased pressure within the pouch and not from the carbohydrates or their osmotic action, for it usually disappeared promptly when the pouch was allowed to drain and it could be produced by the introduction under similar circumstances of 10 per cent NaCl or 0.9 per cent NaCl into the pouch.

Quigley and Hallaran inhibited gastric motility by introducing carbohydrate solutions into the duodenum. Their experiments were indicative regarding the site of initiation of the inhibitory factor, but not conclusive for escape of the solution from the upper intestine was not prevented. Under conditions free from this objection (employing dogs with obstructed pylorus or with a pouch of the entire stomach) we introduced 100 cc. 6 per cent dextrose or 11 per cent lactose or sucrose into the upper jejunum or duodenum and produced typical inhibition of the stomach. Similar injections of 0.9 per cent NaCl did not alter gastric motility.

CONCLUSIONS. Gastric inhibition produced by the ingestion of carbohydrates is due to their presence in the upper intestine (probably to contact with the mucosa). It occurs when such solutions are limited to the upper intestine, but not from carbohydrates restricted to the stomach or, according to Quigley and Hallaran, to the lower ileum or colon. It is apparently not only specific in site of origin but also specific in action, for the latter investigators have shown that it does not inhibit other portions of the gut such as the lower ileum or colon.

Carbohydrate inhibition may normally result from a humoral factor, a nervous factor, or both. Its occurrence in the denervated auto-trans-

planted pouch must depend exclusively on the humoral mechanism and extrinsic nerves are not involved when the reaction occurs in the vagotomized, splanchnicotomized, celiac ganglionectomized stomach. The only indications of dependence on a nervous reflex adduced from this investigation relates to the earlier development of inhibition and with dilute solutions of carbohydrates, the slightly greater effectiveness in the normal stomach than the denervated pouch. However, these differences might result from reduced susceptibility of the denervated tissue to the humoral factor. The humoral factor resembles an inhibitory hormone, i.e., it is chalone-like in origin and action. The similarity of the mechanisms involved in gastric inhibition from ingested fats to that induced by carbohydrates is pronounced, especially in regard to the blood borne factors. The normal control of gastro-intestinal motility by other humoral factors yet unidentified is suggested.

SUMMARY

Carbohydrates entering the normal empty stomach inhibit hunger contractions. This phenomenon occurs in the denervated stomach and is definitely the result of a humoral factor (not carbohydrate per se) produced by the presence of carbohydrate in the upper intestine, but not in other portions of the gut. A nervous reflex, if also involved, is likewise initiated from the same region. The inhibition persists until the onset of digestive contractions, an interval which is short following ordinary ingestion of carbohydrates, but with concentrated solutions may persist for several hours. It apparently is physiologically significant in controlling the rate at which carbohydrates will enter the duodenum.

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AN ECONOMY OF WATER IN RENAL FUNCTION REFERABLE TO UREA

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The results of a study in this laboratory of the quantities of water required for the removal in urine of urea and of several salts were published several years ago (1). The plan of the experiments was extremely simple. The animals used were rats. Urea or salt was added to a basal maintenance diet in progressive increments and the intake of the added substance and the accompanying quantity of water drunk by the animals were measured. The water intake was assumed to be applied entirely to the removal of the ingested materials in the urine, the additional factors of water expenditure, which were relatively very small in the presence of the large quantities of material claiming excretion in urine, being disregarded. The results were presented as concentration values for the ingested substance referred to the volume of the water intake and were regarded as approximately describing the actual concentration in the urine of the substance or substances under investigation. The data thus obtained showed clearly that the water requirements for the removal in urine of equivalent osmolar quantities of the several salts studied is closely identical, and that much less water is required for the removal of a corresponding quantity of urea. It was also found that, when mixtures of salts are fed, their individual water requirements remain additive. When, however, mixtures of urea and salt are fed, it was found that much less water is required for the removal of the two substances together in urine than is prescribed by their water requirements as separately determined. This curious finding indicating an economy of water in the excretion in urine of mixtures of materials containing urea was regarded as deserving further description by a more accurate method of study. To this end experiments were undertaken in which the data were obtained directly from the urine. The results of these experiments are presented in this paper.

PLAN OF EXPERIMENTS. The animals used were nearly grown male rats of approximately the same weight, 200 grams \pm 10 grams, and were between three and four months of age. In each experiment the data were obtained* from a single animal confined in a suitable metabolism cage.

Consecutive twenty-four hour collections of urine were obtained and measurements of the daily quantities of food eaten and water drunk were recorded.

The data sought were measurements of the concentration in the urine of substances added singly and as mixtures to the basal diet. To this end two substances were used in each experiment. The experiment was begun by adding one of the substances to the diet. Then in successive periods this substance was progressively, and in the final period entirely, replaced by the other substance. In all of the experiments, except those in which glucose was studied, the sum of the osmolar values of the substances added to the diet remained the same throughout the experiment. Each period occupied five days, the measurements being obtained from urine collected over the last three days.

The basal diet used was of the purified type, being composed of casein, milk fat, corn starch, yeast and a salt mixture.¹ Since each experiment extended over a number of weeks it was necessary that the basal diet be

¹ The level of protein intake used was lower than in the preceding study (1), the composition of the basal diet being as follows:

Casein.....	8
Yeast.....	3
Salt mixture.....	3
Starch.....	71
Milk fat.....	15

The method of adding the substances under study to this basal diet is described in detail in the preceding paper (1), the essential point being that the carbohydrate-fat ratio was so altered, in terms of the weight quantities of the substances added, as to provide the same caloric value per gram of food eaten in all of the periods of an experiment. With this plan calculation of intake data from a measurement of the weight of food eaten is much simplified and a stationary basis for observation of the acceptance of the experimental diets by the animals is provided. The increase of fat, according to the varying amounts by weight of the substances added, also serves to prevent powderiness of the food and the consequent hazard of scattering, a detail of much importance. An illustration of the adjustment of the carbohydrate-fat factors is given in footnote 4. In the experiments in which the weight of the added substances was relatively small, more of fat and correspondingly less of starch was used in the basal diet in order to provide a suitable texture. The yeast used was dried brewer's yeast which was kindly supplied us by the Northwestern Brewing Company. The salt mixture was prepared by grinding the ingredients together very thoroughly in a mortar. An even distribution of the salts was demonstrated by analyzing samples of the mixture for several of the component substances. The composition of the salt mixture was as follows:

CaCO ₃	32.5
3 MgCO ₃ ·Mg(OH) ₂ ·3H ₂ O.....	7.6
KCl.....	28.0
NaH ₂ PO ₄ ·H ₂ O.....	25.8
Na ₂ CO ₃	5.1
Fe ₂ (NH ₄) ₂ (C ₆ H ₅ O ₇) ₂ ·3H ₂ O.....	1.0

adequate for nutritional maintenance. In this respect it was, however, minimal as regards protein and inorganic substances in order that the quantity of materials presenting for excretion in the urine from the diet should be relatively small and thus interfere as little as possible with the relationship of the added substances to the volume of the urine, an approximate definition of this relationship being the objective of the experiments. The ideal experiment would provide urine containing only the substances under investigation. The extent to which this goal is approached was, in most of the experiments, described by determining the total concentration of substances in the urine by measurement of the freezing point depression. The added materials were found to constitute above eighty per cent of the total excretion of substances.

The significant data are the measurements of concentration in the urine and other values derived from them. Their relationships are best displayed by the graphic method and except for the data obtained from the creatinine experiments, are so presented. In order to illustrate clearly the plan of the experiments, the directly measured data from a sodium chloride-urea experiment are recorded in table 1. Besides the urine data, the measurements of food and water intake and of urine volume are given, and will serve to indicate the regularity of the animal's behavior as regards acceptance of the diet and adjustment thereto of the water intake.

Obviously in a study of the relationship of urine volume to the removal of substances by the kidney, the values for the substances should be expressed in terms of their osmotic activity. Statements of concentration, in the case of the salts, should therefore define the sum of the concentrations of their osmotically active components. The urine concentration data recorded in the charts and tables are, accordingly, osmolar values. They are derived from direct measurement of one of the radicles of a substance. It is assumed that this radicle is accompanied by a complete equivalence, in terms of valency, of the other radicle or radicles of the ingested substance and, also, that dissociation in the urine is complete. The plan of study also prescribes that the replacement of the intake of one substance by another be performed in terms of osmolar equivalence. This requirement will perhaps justify the form of statement, "os-millimols" per gram of food, which is used to describe the additions of substances to the diet.²

² A few illustrations will serve to make these statements clear. The quantity of the non-electrolyte urea added to 1 gram of food in order to provide an intake of one os-millimol per gram is 60 mgm. (1 millimol). This quantity for NaCl is 29 mgm. ($\frac{1}{2}$ millimol) and for Na_2SO_4 is 47 mgm. ($\frac{1}{3}$ millimol). Correspondingly, the osmolar values for urea, NaCl, and Na_2SO_4 in the urine are obtained by multiplying the molal concentrations found by 1, 2 and 3 respectively.

Chemical methods. The methods of analysis by which the data from the urine were obtained are as follows: *Sodium* by uranyl zinc acetate precipitation as described by Butler and Tuthill (2), *potassium* by Fiske's modified cobaltinitrite method (3), *chloride* by Fiske and Lin's method of wet ashing with nitric acid and Volhard titration (4), *phosphate* by the method of Fiske and Subbarow (5), *sulfate* by the benzidine method of Fiske (6), *creatinine* by the method of Folin (7), *glucose* by titration with Benedict's copper solution and *galactose* by the same method after establishing 63.5 mgm. of galactose as the quantity which will reduce 25 cc. of the

TABLE 1

Basal data from a sodium chloride-urea experiment

The measurements from the urine and other values derived from them are graphically presented in the right hand section of figure 4.

PERIODS (5-DAY)	SUBSTANCES ADDED TO BASAL DIET	FOOD INTAKE	WATER INTAKE	URINE	CONCENTRATION DATA FROM URINE (OSMOLAR)			
					Total from Δ	NaCl	Urea	NaCl + urea
I	os-m-mol. per gm. NaCl, 2.0	gm. per day 14.3	gm. per day 44	cc. per day 36.0	1.04	0.87	0.10	0.97
II	NaCl, 1.80 Urea, 0.20	13.1	35	29.5	1.30	0.98	0.24	1.22
III	NaCl, 1.60 Urea, 0.40	12.3	30	22.0	1.54	1.00	0.37	1.37
IV	NaCl, 1.40 Urea, 0.60	12.4	28	21.5	1.70	1.02	0.53	1.55
V	NaCl, 1.10 Urea, 0.90	13.8	26	19.0	1.93	0.92	0.81	1.72
VI	NaCl, 0.80 Urea, 1.20	14.3	25	18.5	2.14	0.79	1.10	1.90
VII	NaCl, 0.40 Urea, 1.60	12.5	23	16.5	2.18	0.50	1.45	1.95
VIII	Urea, 2.00	12.1	22	14.0	2.24	0.25	1.61	1.86

copper solution. The depression of freezing point was measured by means of a Hortvet cryoscope. The values for total ionic concentration were obtained by dividing the observed Δ by 1.86.

Salt experiments. The concentration values found in the urine in a series of experiments in which the chlorides and the carbonates of sodium and potassium were studied are recorded in figure 1. The level of addition of the salts to the basal diet was 2 os-millimols per gram of food. As

may be seen in the graphs, in each of the experiments, as one salt is replaced by another, their osmolar concentrations in the urine rise and fall respectively with a close reciprocity which produces a nearly stationary value for the sum of the component electrolytes of the two salts. This value is also roughly the same in each of the three experiments which were carried out with different animals, and is in the neighborhood of 1.0 osmolar.

Experiments were next undertaken in which an addition of sodium chloride to the basal diet was gradually replaced by a phosphate (KH_2PO_4) or by a sulfate (Na_2SO_4). Here an obstacle was encountered consisting of disturbance of gastro-intestinal function when phosphate and, in less degree when sulfate, were ingested to the extent of 2 os-millimols per gram

Concentration of electrolytes in urine following ingestion of mixtures of salts.

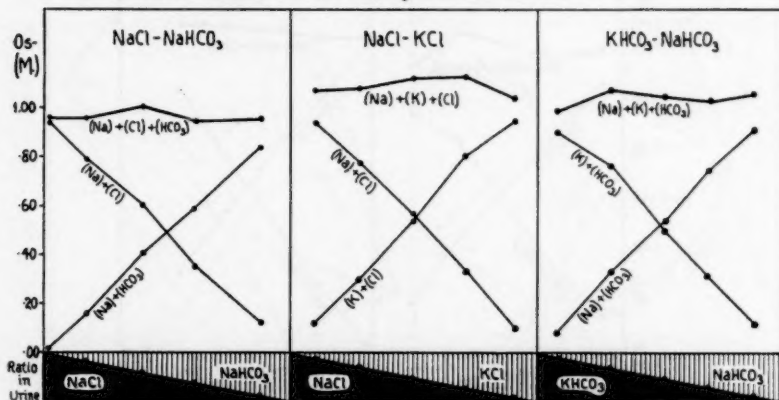


Fig. 1

of food. To avoid this interfering circumstance, it was necessary to carry out the experiments at a level of 1 os-millimol of added substances per gram of food. The measurements obtained from the urine are recorded in figure 2 and are of the same character as those found for the mixtures of chlorides and carbonates. The value for the sum of the concentrations of the radicles derived from the ingested salts is roughly stationary in the presence of extensive change in the concentrations of the component factors. It will be noted that this addition value is approximately 1.2 osmolar instead of 1.0 osmolar found in the chloride-carbonate experiments. Obviously however this higher value cannot be referred to the phosphate or sulfate radicles since it obtains in the initial periods in which the added material is entirely sodium chloride. The data to be presented in the next section will indicate that this appreciably higher level of con-

centration of the electrolytes is referable to a larger relative value for urea permitted by the lower level of intake of salts. Without entering further into this explanation here, it may be mentioned that actual values found for the concentrations of urea in the urine in the presence of additions of 1 os-millimol and of 2 os-millimols of sodium chloride per gram of food were 0.23 osmolar and 0.13 osmolar respectively.

It is the evidence of these experiments that the water requirement for the removal in urine of the six electrolytes studied, Na, K, Cl, HCO_3 , H_2PO_4 and SO_4 , is the same, or at least approximately the same, for each of them and that their individual requirements are directly additive when mixtures of them enter the urine. In view of their widely different con-

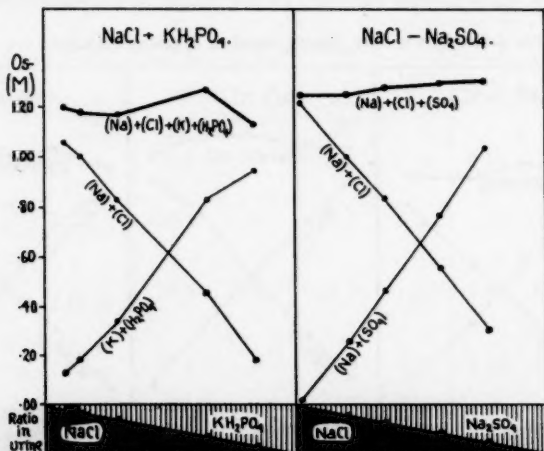


Fig. 2

centration values in the blood plasma, it is interesting that they are, in this respect, identically dealt with.

Urea-salt experiments. In these experiments a much higher level of addition of materials to the food, 5 os-millimols per gram, was successfully used. In order to obtain a detailed description of the finding roughly uncovered by the experiments published several years ago (1), the transition from the initial period when urea alone was added to the food, to the final period in which urea was completely replaced by salt, was accomplished in eight successive steps. Data were thus obtained from six intermediate periods during which urea and salt entered the urine together in differing relative amounts. Two such experiments were carried out, the salt used in one of them being sodium chloride and in the other, potassium chloride. The data obtained were nearly identical. Those from the urea-

sodium chloride experiment are presented in figure 3. In this figure, and in those which follow it, the solid line curves are constructed from the concentration values directly measured in the urine. The points on the broken line curves are the values which would obtain if the individual water requirements of the substances were completely additive when mixtures of them are excreted. These values are calculated from a value for the concentration of urea in the urine when unaccompanied by sodium

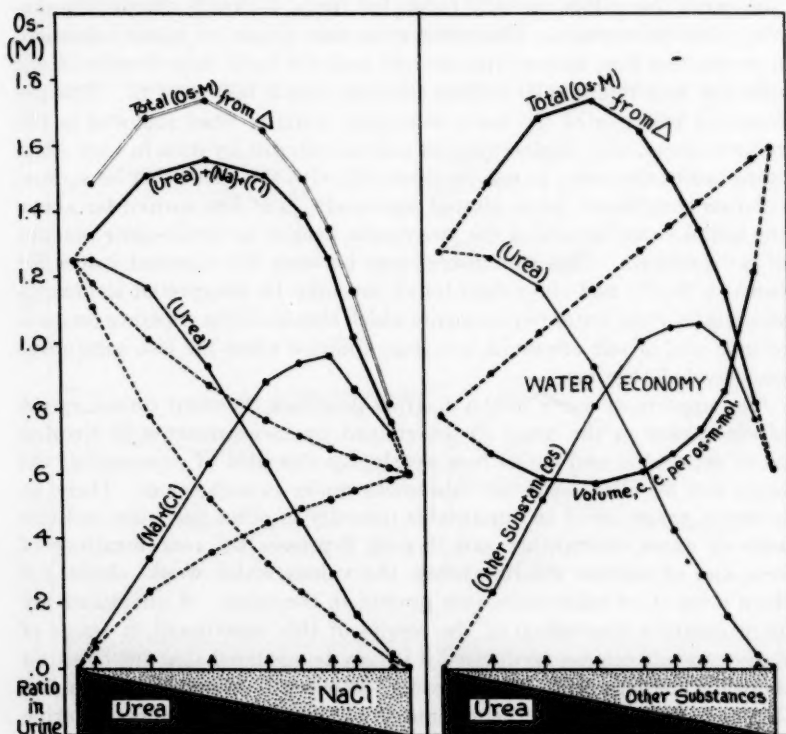


Fig. 3

chloride, a value for sodium chloride when unaccompanied by urea, and the relative amounts of the two substances found in the urine. It will be noted that in the first period of the experiment, when the added substance was entirely urea, the urine contains a small amount of sodium chloride which derives from the basal diet and that in the final period when salt alone was added to the food there is unavoidably a small excretion of urea. It was therefore necessary to obtain theoretical values for the excretion of urea alone and of sodium chloride alone by projection of the

curve found for (urea + NaCl). As may be seen in the left hand section of the diagram, the values thus derived are 1.25 osmolar for urea and 0.6 osmolar for sodium chloride. It would therefore be expected that, as sodium chloride replaces urea in the urine, the sum of the concentrations of the two substances would progressively decrease. The expected path of descent of this value is shown by the broken line curve between the initial urea and the final sodium chloride value. As shown by the solid line curve the values actually found for (urea + NaCl) extensively disobey this expectation. The curve even rises above its initial value and over the first four intervening periods, and not until three-fourths of the urea has been replaced by sodium chloride, does it fall below it. This unexpected behavior of the curve is equally striking when followed in the reverse direction. Replacement of sodium chloride by urea in very small steps causes the curve to rise precipitously with the result that its sodium chloride component, when plotted separately, is at first carried far above the initial value in spite of the progressive decline in the absolute amount of salt excreted. The wide discrepancy between the expected values for (urea + NaCl) and the values found can only be interpreted as demonstrating that the water requirements which obtain for the separate removal of urea and of salt are much less than additive when the two substances are excreted together.

The uppermost curve in the diagram describes the total concentration of substances in the urine as determined by measurements of freezing point depression and shows how nearly, by this plan of experiment, the urine can be filled with the substances under investigation. There is, however, excretion of an appreciable quantity of other materials and this adds to water expenditure and thereby depresses the concentrations of urea and of sodium chloride below the values which would obtain for them if no other substances were present in the urine. A quantitatively more accurate description of the results of this experiment in terms of water expenditure can be devised if it may be assumed that the effect on the water requirement which the data disclose is referable to urea and that the individual water requirements of the other substances in urine have the same value as found for sodium chloride. In other sections of this paper the first of these assumptions is supported and the second one is found to be approximately valid. Permission to relate an urea effect to the total concentration of substances is thus provided. The data from the experiment are plotted in these terms in the right hand section of figure 3 which also contains a curve directly describing water expenditure. Concentration being the reciprocal of volume, a statement of urine volume as cubic centimeter per os-millimol of material can readily be obtained from the measurements of total concentration. The curve thus derived (recorded on the same ordinate scale as the concentration data) is sup-

plied by projection with an initial and a final value and the broken line curve connecting them describes the volume of the urine for the intervening periods which a complete addition of the individual water requirements of the substances would prescribe. The space between the two curves measures the economy of water found when urea and other substances are excreted together. It is, as may be seen in the diagram, a considerable quantity, amounting at its widest to almost one-half of the expected expenditure of water.

Galactose experiments. It was next undertaken to learn whether or not

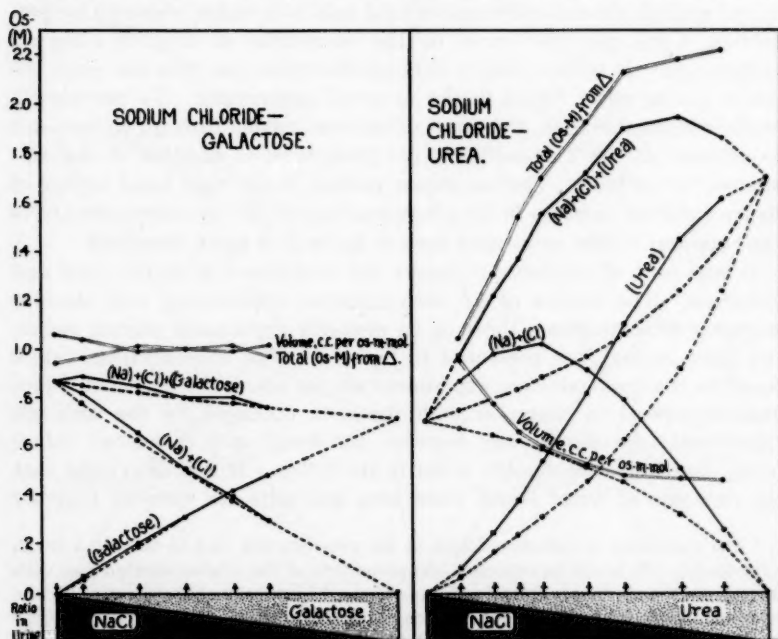


Fig. 4

the removal in urine of other non-electrolytes together with salt is accomplished with the saving of water found for urea-salt excretion. Experiments with galactose were suggested by the observation by Cori (8), that when galactose is fed to rats in large amount about one-half of it is excreted in the urine. The results of a sodium chloride-galactose experiment are recorded in the left hand section of figure 4. The materials were added to the food in amounts intended to provide an excretion of them in the urine to the extent of 2 os-millimols per gram of food. To this end the galactose fraction was doubled in the food in order to allow for the

expected oxidation of about one-half of it.³ Owing to this adjustment and to the large size of the galactose molecule, there was not sufficient space in the diet to permit carrying the experiment to completion at the 2 os-millimol level.⁴ The data obtained from the six periods of the experiment are, however, sufficient to describe clearly the independence of the concentration of sodium chloride and galactose in the urine. Galactose is evidently excreted at a slightly lower level of concentration than is sodium chloride. The curves of their individual values are nearly straight lines and these follow closely the theoretical points calculated from the initial sodium chloride value and a final galactose value, obtained by projection of the galactose curve, on the assumption of additive water requirements. It is thus clearly evident that galactose does not exert the water saving effect found in the urea-salt experiment. To provide directly comparable data, the same animal was carried through an urea-salt experiment at the 2 os-millimols per gram level of addition of the substances to the food. The results are plotted in the right hand section of figure 4 and the increase in the concentrations of the two substances above the expected values, as already seen in figure 3, is again described.

It was next of interest to observe the excretion together of urea and galactose. The results of an urea-galactose experiment, and also for purposes of comparison, those of an urea-salt experiment carried out on the same animal, are presented in figure 5. The concentration values found in the urea-galactose experiment are far above the expected values and correspond in character with the data produced by the urea-salt experiment, the discrepancy between the found and calculated values being, however, considerably wider in the latter. It is thus evident that the economy of water found when urea and salts are excreted together

³ The quantities of galactose found in the urine showed this to be only a rough adjustment. As would be expected, the proportion of the intake excreted rose with increase in the amount ingested. For instance, the excretion: intake ratios for the successive periods of the sodium chloride-galactose experiment (fig. 4) were, beginning at the second period, 0.33, 0.54, 0.62, 0.63, and 0.69.

⁴ The composition of the diet in the final period of the galactose-urea experiment which provided 0.75 os-millimol urea and 1.25 os-millimol galactose per gram of food was as follows:

Urea.....	4.5
Galactose.....	45.0
Protein.....	8.0
Yeast.....	3.0
Salt mixture.....	3.0
Starch.....	3.2
Milk fat.....	33.3

As may be seen the limit of adjustment of the fat-carbohydrate factors is reached and a further addition of galactose would cause a decrease of the caloric value of the food and thereby raise the level of intake of the other factors in the basal diet.

is also found, although to a somewhat less extent, when the non-electrolyte galactose is excreted with urea. The concentration relationships found when both sodium chloride and galactose accompany urea are recorded in figure 6.

The results of these experiments make it clear that, among the substances studied, the water saving effect is referable only to urea.

Glucose experiments. In these experiments, glucose excretion in the

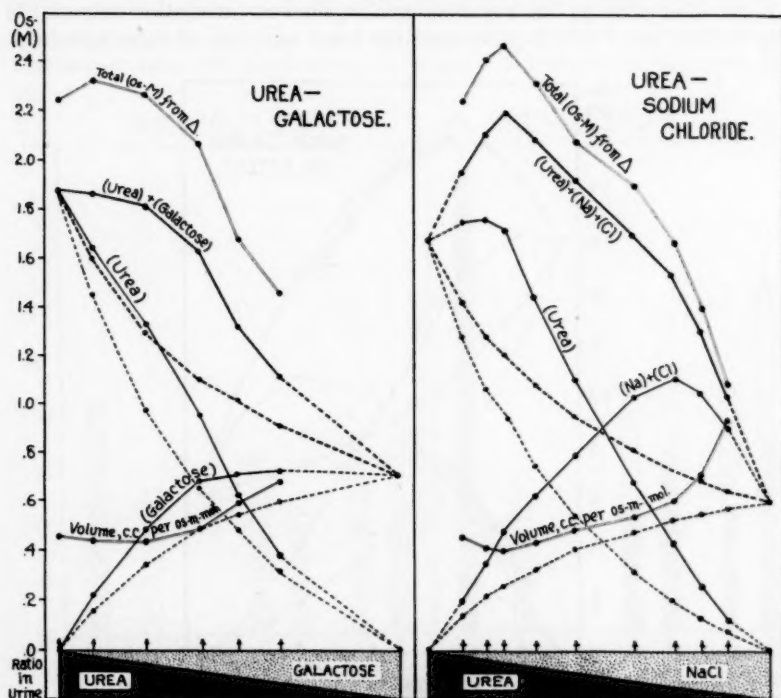


Fig. 5

urine was produced by subcutaneous injections of phloridzin. The plan of step-wise replacement of one substance by another offered obvious difficulties and was not attempted. Instead, three periods were used. In the first, with the animal on the basal diet, an injection of 2.0 cc. of a 10 per cent emulsion of phloridzin in olive oil was given and the urine collected over a period of two days. It was found that this quantity of phloridzin gave a glucose concentration in the urine of between 0.5 M and 0.6 M, and that larger dosage did not dependably produce a higher concentration. The maximal effect of the drug was found to persist for

two or three days and then to decline rapidly. After an interval of eight to ten days, sodium chloride was added to the basal diet to the extent of 2 os-millimols per gram and the urine collected over the last two days of a five-day period. This intake of salt produced an excretion which was roughly equivalent to the quantity of glucose excreted during the phloridzin period. Then, continuing the salt intake, the animal was again phloridzinized and a two-day collection of urine obtained. The same steps were followed in carrying out a glucose-urea experiment on another animal. It is evident that, in this procedure, the total quantity of substances con-

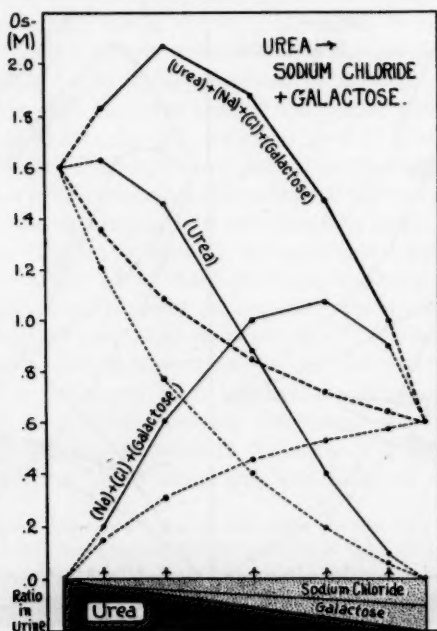


Fig. 6

veyed into the urine during the last period is approximately double the quantity excreted in each of the preceding periods. This, however, does not constitute a serious defect in the experimental plan. It should be remembered, as an essential premise of this study, that increments of materials to be excreted in the urine produce accurate adjustments of water intake with the result, for instance, that the concentration of sodium chloride found in the urine when 2 os-millimols of NaCl per gram of food are ingested is not appreciably altered by increasing the intake to 4 os-millimols per gram.

The results of a sodium chloride-glucose experiment are described by the diagrams in the left hand section of figure 7. The first column represents the concentration of sodium chloride found in the urine when 2 osmillimols per gram were added to the basal diet, and the second column measures the glucose concentration when the animal was phloridzinized while receiving only the basal diet. The two columns marked *F* represent the sum of the concentrations found in two consecutive twenty-four-hour urine specimens, collected after phloridzinizing the animal while on the diet with added salt. The two columns marked *C* define expected values calculated from the concentrations found for sodium chloride and for

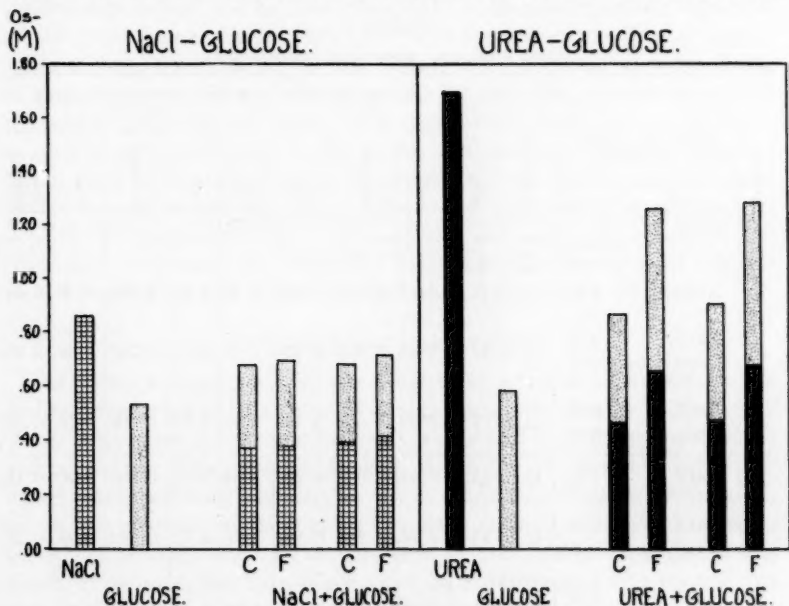


Fig. 7

glucose in the first and second periods of the experiment respectively and the ratio of the quantities of the two substances excreted during the final period. As may be seen, the found and calculated values agree quite closely indicating that the individual water requirements for the removal of sodium chloride and of glucose in urine remain additive when they are excreted together. In other words, glucose when excreted with salt does not exert the water saving effect found for urea.

In the right hand section of figure 7 the columns describe the results of an urea-glucose experiment. Here the found values for the sum of the concentrations of glucose and of urea in urine are extensively above the

values calculated on the basis of additive water requirements and thus indicate a considerable economy of water expenditure when glucose and urea are excreted together.

Creatinine experiments. Since galactose and glucose are excreted in the urine only under unusual circumstances, it seemed desirable to investigate the behavior of one of the non-electrolytes which are regularly present. These, excepting urea, are very small factors in the total concentration of substances in the urine. Among them creatinine is quantitatively the most prominent and was for this reason, and also because of its availability, selected for study. It was found that a high level of intake

TABLE 2
Data from two creatinine experiments

PERIOD	SUBSTANCES ADDED TO DIET		FOOD EATEN		DAILY EXCRETION IN URINE			CONCENTRATION DATA URINE, OSMOLAR				
					NaCl	Creatinine	Urea	Total from Δ	NaCl	Creatinine	Urea	Urea Total
		os-m-mol. per gm.	gm. per day	cc. per day	os-m-mol. per gm. food							
1	NaCl,	1.0	10.8	12.2	1.21	0.005	0.226	1.47	1.07	0.005	0.20	0.14
2	NaCl, Creatinine,	0.5 0.5	12.2	12.5	0.68	0.317	0.410	1.60	0.67	0.310	0.40	0.25
3	NaCl, Urea,	0.8 0.2	11.8	9.4	0.93	0.005	0.422	1.99	1.17	0.006	0.53	0.27
1	NaCl,	1.0	13.5	15.0	1.13	0.006	0.222	1.34	1.02	0.005	0.20	0.15
2	NaCl, Creatinine,	0.5 0.5	13.2	13.8	0.76	0.253	0.502	1.76	0.73	0.242	0.48	0.27
3	NaCl, Urea,	0.7 0.3	12.7	11.0	0.98	0.005	0.528	1.98	1.13	0.005	0.61	0.31

could not be used; when the food contained more than 0.5 os-millimol of creatinine there was disturbance of gastro-intestinal function as evidenced by loose stools. The experiments were carried out in three periods of five days each, the measurements being obtained from urine collected over the last three days of each period. During the first period sodium chloride was added to the basal diet to the extent of 1 os-millimol per gram of food. In the next period one-half of the sodium chloride was replaced by creatinine and in the final period a smaller quantity of urea was substituted for its equivalence of sodium chloride, for a reason which will be presently apparent. The results obtained from two experiments are given in table 2. It may first be noted that the values found for the total

concentration of substances in the urine, as derived from measurements of Δ , describe a considerable rise when one-half of the sodium chloride added to the basal diet in the first period is replaced in the second by creatinine. This event suggests that creatinine exerts an effect on concentration similar to that found for urea. This inference is obstructed by the values found for urea. During the creatinine-sodium chloride period, the concentration of urea in the urine is double the value found in the sodium chloride period and the data for the excretion of the substances, per gram of ingested food, show a large increase of urea excretion.⁵ The increase in the total concentration of substances observed in the second period is therefore presumably caused, not by creatinine, but by the accompanying extension of the urea factor. In the third period of the experiment, it was undertaken to obtain approximately the (urea): (total substances) value found in the creatinine-sodium chloride period by replacing a small portion of the sodium chloride with urea. This attempt was roughly successful and, as may be seen in the table, a rise in the total concentration of substances in the urine is obtained which is even more extensive than the rise found in the creatinine-sodium chloride period. From these data it may be dependably concluded that creatinine does not exert the concentrating effect found for urea. The data also suggest that creatinine itself submits to the urea effect but to a less extent than does sodium chloride.

SUMMARY AND CONCLUSIONS

The water requirements for the removal in urine of a number of substances were studied in a series of experiments with rats by placing relatively large amounts of the substances, singly and in mixtures, in the food and measuring their concentrations in the urine. It was found that the water requirements established for the individual substances remain additive when mixtures of them enter the urine, except when urea is a component of the mixture. In the presence of urea, water expenditure was found to be much less than the sum of the requirements for urea and the accompanying substances as separately determined.

⁵ From the data in table 2 it is evident that almost one-half of the ingested creatinine fails to appear in the urine. The possibility that the increase in urea excretion derives from destruction of creatinine is therefore strongly suggested. From the data in the table it may be calculated that in the second period of the first experiment the increase of urea N in the urine is 5.2 mgm. per gram food eaten and that the deficit for creatinine N is 7.9 mgm. per gram food eaten. In the second experiment these values for urea N increase and creatinine N deficit are 7.8 mgm. and 10.6 mgm. respectively. It cannot be dependably inferred that the creatinine deficit in the urine is caused by a destruction of creatinine within the body because of the possibility of failure of complete absorption of the creatinine from the gastro-intestinal tract, and of an absorption of urea derived from creatinine by the action of intestinal bacteria.

These results describe an economy of water in the secretion of urine which, among the substances studied, is referable only to urea. The substances normally present in urine which were examined in this respect, were the electrolytes Na, K, Cl, HCO_3 , H_2PO_4 and SO_4 , and the non-electrolytes urea and creatinine, and taken together they constitute about 95 per cent of the total of materials presenting for excretion. A quantitatively significant relationship of one or more of the relatively very small factors of urine composition, which were not investigated, to the water saving observed in these experiments is therefore improbable. It was also demonstrated that the non-electrolytes, galactose and glucose, which may under unusual circumstances enter the urine, do not exert the effect found for urea.

An economical use of water is, for terrestrial animals, a conspicuous necessity. It is therefore an interesting instance of the fitness of biological substances that the largest "waste product" in urine incidentally performs an important service to the organism. An explanation of this effect by urea is not at hand. Presumably it should be sought in terms of various physical properties which urea exhibits to degrees which are almost unique.

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INFLUENCE OF THE PANCREAS AND THE LIVER UPON THE DEXTROSE TOLERANCE CURVE¹

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The prevailing conceptions as to the mechanism of the so-called normal dextrose tolerance curve may be briefly summarized as follows:

1. The administration of dextrose normally stimulates the pancreas to an increased secretion of insulin (1), (2), (3), (4), (5), (6), (7), (8).

2. This increased amount of insulin in the circulation accounts for the normal dextrose tolerance curve by bringing into play increased storage and oxidation phenomena.

3. The abnormal tolerance curve obtained in diabetes mellitus and in the depancreatized animal is due to the lack of pancreatic response to the administered sugar.

4. The "diabetic" type of curve obtained in starvation is due to the lack of response from a normal pancreas. Even after long fasting, however, a preliminary dose of sugar will render the subsequent tolerance curve more nearly normal. Hence the pancreas requires continual stimulation in order to be able to respond normally (1), (5), (9), (10).

5. Since similar abnormal tolerance curves are also obtained on exclusive fat or protein diets, the "diabetic" response in starvation is due to the lack of carbohydrate per se rather than to the lack of nutrition (9), (11), (12), (13).

It occurred to us that one might test the fundamental basis of the above concepts by substituting a constant source of insulin for the pancreas, in the depancreatized dog. Such an animal while receiving, from a constant injection pump, a steady supply of insulin sufficient to maintain a constant blood sugar level (and being, to that extent, a normal animal) would not be able to mobilize an additional supply of insulin when sugar was administered. If, therefore, the response of the pancreas were a major factor in determining the normal tolerance curve, such animals should give

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abnormal curves. Beginning with such experiments, we were led by our results to investigate the influence of the liver upon the dextrose tolerance curve. For this purpose we performed dextrose tolerance tests upon hepatectomized dogs which were receiving a constant glucose injection sufficient to maintain a constant blood sugar level. Finally, in an effort to clarify the meaning of our results, we performed dextrose tolerance tests on normal and depancreatized animals from which blood samples were taken simultaneously from the carotid artery and the femoral, portal and hepatic veins.

I. PANCREAS. *Method.* Depancreatized dogs were prepared in one of two ways. Certain of the experiments were performed on animals which had recovered from the operation, with well healed and non-infected wounds and which had been maintained on a meat—raw pancreas—sugar diet and insulin. These animals were trained to lie quietly on an animal board while the injections and blood sampling were done by means of venous puncture with hypodermic needles. Other experiments were performed on dogs anesthetized with Pento-Barbital Sodium (Abbott)—about 25 mgm. per kgm. body weight intravenously. After being anesthetized these animals were depancreatized and the necessary blood vessels exposed and cannulated. The entire experiment was then performed on the anesthetized animal, additional anesthesia being administered when necessary. The animal was sacrificed at the end of the experiment. In both types of preparation all food and insulin were withheld for 18 hours previous to the experiment.

In most of this work the constant intravenous injections of insulin or insulin and dextrose required to maintain constant blood sugar levels were made with the apparatus previously described by one of us (S. S. (14)). In some of the earlier experiments the oil-capillary method employed by Burn and Dale (15) was used. The necessary amounts of insulin and dextrose varied in different animals and ranged from $\frac{1}{175}$ to $\frac{2}{3}$ unit insulin per kilogram body weight per hour and from $\frac{1}{15}$ to $\frac{2}{3}$ gram dextrose per kilogram body weight per hour. The smaller amounts of insulin and of dextrose were required by the anesthetized animals, while the larger amounts were necessary for the trained unanesthetized animals. The exact rates of injection required for each animal were determined by the method of trial and error in each case. We have evidence to show that, once established, constant blood sugar levels can be maintained for as long as 8 hours by continuing the same constant injections. Where no constant blood sugar level could be established, the experiment was interrupted or the results discarded. However, all experiments in which constant levels were obtained have been reported without selection on the basis of the results obtained. When a constant blood sugar level was obtained, it was observed

for at least 1 to 2 hours before the dextrose tolerance test was started, and the established rates of constant insulin and dextrose injection were very carefully maintained throughout the experiment.

To avoid possible differences arising from varying rates of absorption from the gastro-intestinal tract, the dextrose tolerance tests were always performed by the intravenous route. Except where noted, 1.75 gram dextrose per kilogram body weight was used. "True blood sugar" values were determined by the Somogyi modification of the Shaffer-Hartmann method (16).

Results. The results are graphically summarized in figures 1 to 8. The experiments are divided into groups according to the procedure followed. Each figure represents a different group. The shaded areas in the figures represent the spread of the highest and lowest blood sugar values obtained in all the experiments of the particular group. The heavy central lines represent mean values.

Group 1 (control). Slightly modified normal dextrose tolerance curves were obtained in normal animals which had been anesthetized with Pento-Barbital Sodium and subjected to a control laparotomy within two hours previous to the test. The dextrose tolerance curve obtained in normal unanesthetized dogs is sufficiently well known and need not be described here. It may be seen that in this group the blood sugar levels have returned to or below the pre-test level within two hours. The rather high blood sugar values attained in the first 15 to 30 minutes of these tests may be partly accounted for by the anesthesia but are probably chiefly due to the fact that the glucose was administered intravenously.

Group 2 (control). Typically "diabetic" dextrose tolerance curves were obtained in chronically depancreatized, unanesthetized dogs.

Group 3 (control). Distinctly "diabetic" dextrose tolerance curves were obtained in anesthetized dogs from which the pancreas had been removed within two hours prior to the test. When these curves are compared with those of groups 1 and 2, it is evident that within two hours after the removal of the pancreas there is no store of available insulin in the tissues sufficient to appreciably influence the tolerance curves.

Group 4. These are the dextrose tolerance curves obtained in anesthetized and acutely depancreatized animals in which we attempted to maintain a constant normal blood sugar level by the constant injection of insulin. It was almost impossible to determine the exact constant injection of insulin necessary in each experiment to keep the blood sugar at a constant level. It may be seen that in most of these experiments the blood sugar level was slowly declining before the test glucose was injected. In spite of this, however, most of the curves are "diabetic" in character, although not so markedly so as those of group 3. But it should be noted that some of these tests

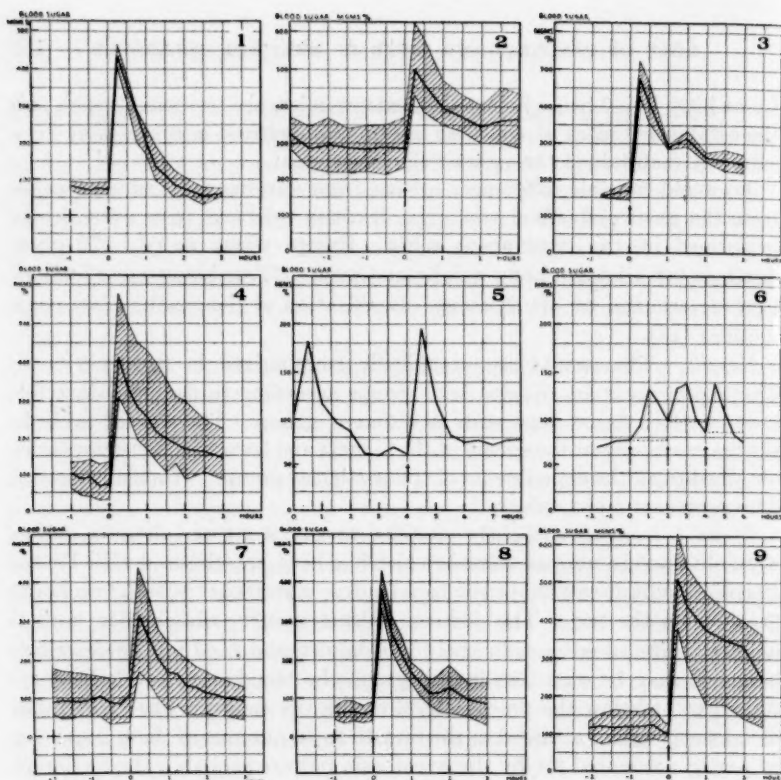


Fig. 1. Control dextrose tolerance tests in anesthetized normal dogs. The arrow indicates the administration of the test dose of sugar. The shaded area represents the spread of the highest and lowest blood sugar values obtained in all of the experiments of this group. The heavy central line represents the mean values. The same conventions are observed in the remaining figures.

Fig. 2. Control tests in chronically depancreatized dogs where no anesthesia was used.

Fig. 3. Control tests in anesthetized, acutely depancreatized dogs.

Fig. 4. Dextrose tolerance tests in anesthetized, acutely depancreatized dogs, receiving a constant injection of insulin.

Fig. 5. Representative tolerance test in an anesthetized, acutely depancreatized dog, receiving a constant injection of insulin, and previously given $\frac{1}{4}$ of the test dose of sugar.

Fig. 6. Representative experiment in an anesthetized, acutely depancreatized dog, receiving a constant injection of insulin, and to which three successive small doses of dextrose were given.

Fig. 7. Dextrose tolerance tests in chronically depancreatized dogs, where no anesthesia was used, and which were receiving a constant injection of insulin plus dextrose.

Fig. 8. Dextrose tolerance tests in anesthetized, acutely depancreatized dogs, receiving a constant injection of insulin plus dextrose.

Fig. 9. Dextrose tolerance tests in unanesthetized, hepatectomized dogs, receiving a constant injection of dextrose.

closely approached the normal. All these animals, like those in the previous groups, were fasted for 18 hours prior to their tests.

Groups 5 and 6. The corresponding figures illustrate representative experiments from these groups, which show the influence of previous dextrose administration on a subsequent dextrose tolerance curve. This adjustment in metabolic response, which is ordinarily attributed to the activity of the pancreas, is seen to occur in anesthetized and acutely depancreatized dogs, receiving a constant injection of 1/175 unit insulin throughout the experiment.

In the group 5 experiment, the administration of $\frac{1}{4}$ of the test dose of dextrose four hours prior to the test resulted in a subsequent dextrose tolerance curve which is essentially normal. It may be seen that the tolerance curve proper is very little higher than that previously resulting from $\frac{1}{4}$ of the amount of sugar used, and that it stands in marked contrast to most of the curves obtained in group 4.

In the group 6 experiment, 3 successive doses of $\frac{1}{4}$ of the amount of sugar ordinarily used, result in successively better tolerance curves.

Groups 7 and 8. In view of the above results, the dextrose tolerance tests in these groups were performed on chronically depancreatized (group 7) and anesthetized acutely depancreatized animals (group 8) in which a constant blood sugar level was maintained by simultaneous constant injections of both insulin and dextrose. Much less difficulty was experienced in obtaining constant blood sugar levels under these conditions, than by the injection of insulin alone. Most of the tolerance tests yielded normal curves, and in two cases (in group 7) the blood sugar values, $2\frac{1}{2}$ hours after the sugar was administered, were well below the pre-test levels.

Comment. The fact that normal reactions to administered dextrose have been obtained in totally depancreatized animals, under our experimental conditions, is incompatible with the prevailing views of the dextrose tolerance mechanism as outlined above. This vitiates the importance of the hypothesis of Zunz and La Barre (17, 18) to the effect that hyperglycemia stimulates the secretion of insulin from the pancreas by acting upon a centre in the thalamic region of the central nervous system; the stimulation being transmitted through the parasympathetic system. Our results are more in accord with the work of Quigley, Hallaran, and Barnes (19), of Gayet and Guillaumie (20), and of Houssay et al. (21), which shows that the blood sugar level can be efficiently regulated in animals in which the pancreas has been freed from central vagus control and in animals which possess only pancreas grafts. Our results are also contrary to the hypothesis of Sweeney (9), who has postulated an intermediate hormone which is secreted during hyperglycemia and acts upon the pancreas to stimulate the production of insulin.

The data here presented are not incompatible with the recent hypothesis

of Himsworth (22),³ who believes that hyperglycemia is followed by the liberation from the liver of "insulin kinase" which then activates the previously inert insulin which has already been secreted into the blood stream. Our data might also be reconciled with the suggestion of Campbell and Macleod (24), based on the work of Allan (25), namely, that insulin may act according to the laws governing enzyme action. From this point of view, the characteristic normal tolerance curve might be explained as being due to the increased activity of the insulin already present in the circulation upon the greater amount of substrate which appears. These hypotheses, however, are not acceptable in the light of other work. The remarkable work of Houssay and his co-workers (26), in the last several years, has extended our knowledge concerning the important influence of the hypophysis upon carbohydrate metabolism. Stimulated by their results, we have been able to obtain normal dextrose tolerance curves in completely hypophysectomized-depancreatized dogs, which have received no insulin for weeks. These results, which are reported in a subsequent paper (27), confirm the results and conclusions presented here, and furthermore, remove the necessity of accounting for the normal dextrose tolerance curve by the assumption of an insulin activating mechanism, or an increased glucose equivalence of this hormone.

II. LIVER. Since it was evident that the pancreas is not essential to the metabolic reactions which determine the normal dextrose tolerance curve, it became of interest to investigate the rôle of the liver. There are many theoretical and experimental considerations which point to the latter organ as a major factor in this regard. The influence on carbohydrate metabolism of experimentally induced hepatic changes has recently been reported by Althausen and Theones (28).

Method. Dextrose tolerance tests were performed, as described above, in hepatectomized dogs which were receiving a constant injection of dextrose sufficient to maintain the blood sugar level at a constant height. Hepatectomies were performed by the method of Markowitz and Soskin (29, 30). The rate of dextrose injection usually required to maintain the blood sugar of these animals at a constant level was $\frac{1}{4}$ gram per kilogram body weight per hour, as previously determined by Mann and Magath (31).

In another series of animals, constant injections were not made, but dextrose tolerance tests were performed in both normal and depancreatized dogs, under Pento-Barbital Sodium anesthesia, during which blood samples

³ It must be pointed out that Himsworth's conclusions are chiefly based upon curves of arterio-venous blood sugar difference obtained in patients. We are doubtful of the validity of these conclusions since in a more recent communication (23) we have shown that "the arterio-venous blood sugar difference cannot be used as a quantitative estimate of sugar utilization by the muscles, unless it is corrected for rate of bloodflow and hydration of the blood, and is determined at frequent intervals."

were taken simultaneously from the carotid artery and the femoral, portal and hepatic veins. The carotid artery and femoral vein were cannulated. Blood was obtained from the portal and hepatic veins through an abdominal incision by means of a hypodermic needle and syringe. The hepatic vein was approached between the liver lobes.

Results. The results are graphically summarized in figures 9 and 10. Figure 10, in which blood sugar differences are plotted, is accompanied by table 1 which details the actual blood sugar values in this group.

Group 9. These liverless animals, though the pancreas was intact, yielded uniformly and markedly "diabetic" tolerance curves. This occurred in spite of the fact that the blood sugar levels were declining in some cases before the test, a condition which would tend to render the curves more nearly normal.

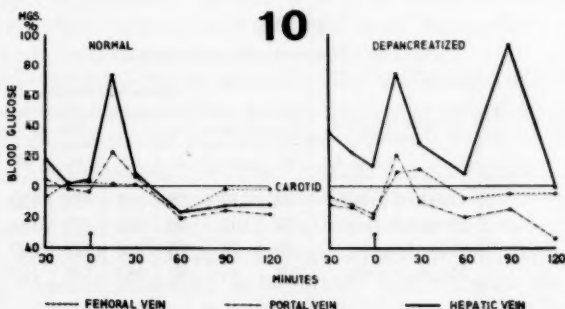


Fig. 10. Four-sample experiments in anesthetized normal, and anesthetized, acutely depancreatized dogs. For purposes of comparison, and in order that the large range of blood sugar variation may not obscure the smaller blood sugar differences, the blood sugar values in the hepatic, portal and femoral veins are plotted as \pm or $-$ differences from the arterial blood sugar level. Arrows indicate the administration of dextrose. (The actual blood sugar values for these tests are given in table 1.)

Group 10 (table 1). A comparison of the results obtained from such "4-sample" tests in normal and depancreatized dogs, shows that, while in the normal animal the hepatic vein blood sugar level falls below that of the arterial blood within about half an hour after the administration of dextrose, the diabetic animal maintains its higher hepatic vein level until the end of the test. It should be noted that, in the normal animal (cf. table 1), when the carotid artery value has fallen to its original level, the hepatic vein value is still below both the carotid artery level and its own pre-test level. Although storage of a portion of the administered sugar by the normal liver may account for some of this difference, it is apparent that the normal liver differs from the diabetic in that the former reacts to an in-

creased sugar supply by a relatively prompt diminution in the amount of sugar which it has been supplying to the blood from its own resources.

Comment. It is apparent that the presence of the normal liver is essential to the normal dextrose tolerance curve and that, when the liver is absent, the intact pancreas and musculature, etc., are unable to produce a normal response.

These results cannot be reconciled with the prevalent idea that the storage and utilization of carbohydrate are the chief or only factors concerned in the normal dextrose tolerance curve. It has previously been shown that the liver is the sole source of blood sugar in the fasting organism (32, 33). The blood sugar level in this condition therefore represents a balance between the sugar leaving the blood on the one hand and the sugar entering the blood from the liver on the other hand. The fact that a more or less

TABLE 1
Group 10 (Four-sample experiments)

DESCRIPTION	SOURCE OF BLOOD	BLOOD SUGAR LEVEL							
		Minutes before test			Minutes after test				
		30	15	1	15	30	60	90	120
Normal	Carotid artery	84	79	78	291	173	130	97	81
	Femoral vein	77	81	80	292	174	113	95	79
	Portal vein	88	77	74	313	179	109	81	63
	Hepatic vein	101	80	82	364	181	113	84	69
Depancreatized. . .	Carotid artery	118	116	123	473	370	317	298	285
	Femoral vein	111	104	105	482	381	309	293	280
	Portal vein	106	102	102	493	360	297	283	251
	Hepatic vein	153	136	136	547	398	325	391	284

constant blood sugar level is usually maintained in the fasting state indicates that these processes ordinarily continue at relatively constant and mutually inter-dependent rates. It is obvious that when the blood sugar level falls it may be due either to an increased loss of sugar from the blood into the tissues or to a decreased entry of sugar into the blood from the liver, or to both. Our results indicate that a decreased output of sugar from the liver is an important factor though not necessarily the only factor in determining the characteristic fall of the blood sugar curve after the administration of dextrose.

SUMMARY AND DISCUSSION. Our results show that:

1. There is no appreciable store of insulin in the tissues, by which the dextrose tolerance test may be affected, once the pancreas is removed.
2. The influence of previous sugar administration on a subsequent dextrose tolerance curve can be demonstrated in the absence of the pancreas.

This is contrary to the general belief that this effect is due to an increased responsiveness on the part of this organ.

3. The supply of sugar available to the organism is an important factor in establishing a constant blood sugar level by the constant intravenous injection of insulin in depancreatized dogs.

4. Providing that there is sufficient circulating insulin to keep the blood sugar level constant under the conditions described above, normal dextrose tolerance curves can be obtained in depancreatized dogs. This is contrary to the hypothesis that the normal curve is determined by a secretion of insulin from the pancreas in response to the incoming sugar.

5. The normal liver is a major factor in determining the normal dextrose tolerance test.

6. In the presence of a sufficiency of insulin, but not necessarily an extra secretion from the pancreas, the normal liver, as one of its responses to administered dextrose, decreases the output of blood sugar, which it has been supplying from its own resources.

Since, from the foregoing, it is unnecessary to assume a mobilization of insulin from the pancreas in order to account for the normal dextrose tolerance curve, the occurrence of the normal tolerance curve cannot henceforth be used as presumptive evidence that such a mechanism exists.

Our results yield a rational explanation for the "diabetic" type of tolerance curve and the associated low respiratory quotient obtained in starvation, and during a dietary regimen of protein and fat, *despite the presence of a normal pancreas*. It is obvious that, although the liver is a major factor in determining the dextrose tolerance curve, it can respond normally to sugar administration only when under the influence of a suitable endocrine balance. This balance, for the sake of simplicity, may be supposed to consist of the opposing influences of the hormones of the pancreas and the hypophysis.⁴ When these glands are exerting their normal and proportionate influences upon the liver, a blood sugar level slightly above the range which we consider normal is an adequate stimulus to the liver for a readjustment of the blood sugar towards the normal range. Under these conditions, a sudden influx of exogenous sugar produces a reaction which we recognize as the normal dextrose tolerance curve. When the endocrine relationships are disturbed, the blood sugar level may far exceed the normal range before it becomes an adequate stimulus for liver readjustment. In the experimental diabetic animal this endocrine balance is upset and excessive gluconeogenesis results from removal of the pancreas (34). In the human diabetic the same consequences may ensue from either a disability of the islands of Langerhans or an excessive activity on the

⁴ We do not wish, at this time, to enter into a discussion of the inter-relationships of this gland with the thyroid and adrenal glands, etc., which undoubtedly play a part in this phenomenon.

part of the hypophysis, etc. An increased gluconeogenesis must also occur (providing no *a priori* assumption of a decreased utilization of sugar is made) in the fasting state and during a diet composed solely of protein and fat, in order that the normal blood sugar level be maintained. Under these conditions, however, the normal endocrine balance still exists. Hence, while repeated sugar administration in the diabetic organism yields repeated diabetic responses, successive dextrose tolerance tests in starvation and similar conditions, produce successively more normal tolerance curves, as the liver (after a comparatively short lag) responds with a decreasing production and outpouring of sugar into the blood. The relation of the excessive gluconeogenesis to the low respiratory quotient, and the coincidental decrease of gluconeogenesis with an increasing respiratory quotient has been discussed by one of us (S.S.) in a previous communication (34).

CONCLUSIONS

The pancreas is not essential to the metabolic reactions which determine the normal dextrose tolerance curve. The presence of the normal liver is essential. The theoretical implications of these results are discussed.

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THE HYDROSTATIC FACTOR IN VENOUS PRESSURE MEASUREMENTS

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It is generally accepted that the pressure in any vein is the result of two factors: the residuum of the head of pressure created by the heart beat, and the influence of gravity. It is also stated that the latter is determined by the height of the column of blood that is present between the point measured and the entrance into the right auricle.

As measurements of venous pressure taken in the feet of standing human subjects are at variance with this statement a study was made of the venous pressure in dogs in the horizontal and two vertical (head-up, head-down) positions. In the initial experiment it was found that the venous pressure-change in the hind foot, on going from the horizontal to the vertical head-up position, was 322 mm. while the hydrostatic column from foot cannula to heart was 445 mm. This indicated, at once, that the heart is not the reference point from which the hydrostatic factor in venous pressure is measured, a conclusion which was confirmed by subsequent experiments. In a dead animal the venous system was found to act as an unbroken column from head to tail with the hydrostatic reference point, which will be referred to as R.P., approximately in the middle of the animal, both in the horizontal and vertical positions. In the living animal the venous system is broken at the heart and must be considered as two columns, each with its own reference point. Each of these points was found to be located a short distance from the heart.

Theoretically, the heart could only be the reference point if the veins closely approximate rigid tubes. Since they have a certain degree of elasticity, which varies with the internal pressure, the reference point may be anywhere in the venous system, depending on the relative elasticity of its different parts. It is therefore theoretically indeterminate and cannot be located except by direct measurement.

In a rigid container, open to the atmosphere (fig. 1a) the pressure at the bottom, p , equals the pressure at the top of the liquid (atmospheric pressure) plus the pressure of the column of fluid h .¹ In such a system hydro-

¹ The pressure of the column of liquid is dgh , where h is the height of the column, d , the density of the liquid, and g the acceleration of gravity, but to simplify the equations h will be used throughout.

static pressures, in excess of atmospheric, are measured from the top of the liquid.

In an elastic tube system filled with liquid, for which we may take as a model a rigid tube with an elastic membrane stretched across each end, the position of R.P., the hydrostatic reference point, may be anywhere in the tube, depending on the relative elasticities of the membranes at the two ends. If these membranes are the same size and elasticity R.P. will be approximately in the middle of the tube, as in figure 1b. If the liquid in the tube is under no pressure except hydrostatic pressure, and a rigid open-end manometer is introduced at the bottom, fluid will rise in this manometer to a height h_z and the top of the fluid in the manometer will measure

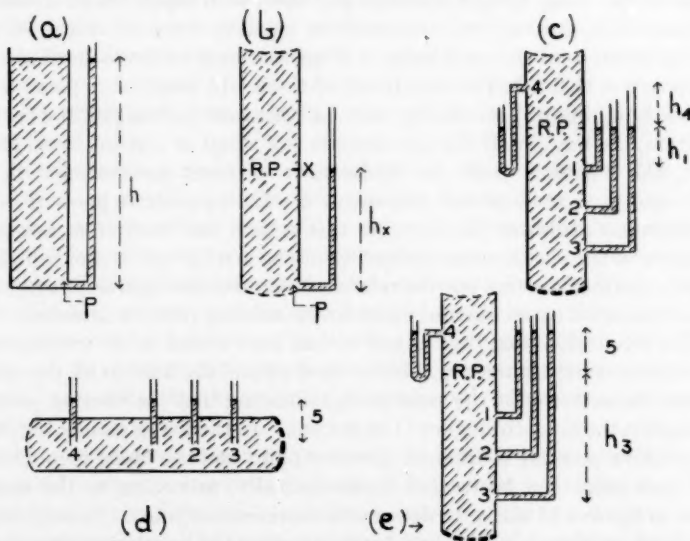


Fig. 1. Position of hydrostatic reference point in rigid and elastic tube systems

the position of R.P. in the elastic tube system, which in this case is also the level of atmospheric pressure in the system, and p , the pressure at the point where the manometer is introduced, is equal to atmospheric pressure plus h_z .

If the membrane at the bottom is more rigid than that at the top R.P. will move toward the top of the tube. In such a system, where R.P. is two-thirds way up a tube 30 cm. long (fig. 1c) fluid in the manometers introduced at 1, 2, 3, 4 will all stand opposite R.P., the hydrostatic reference point. The pressure at any point in this elastic tube system is equal to atmospheric pressure plus or minus the hydrostatic pressure measured to

R.P. ($p_1 = \text{atmospheric pressure} + h_1 = \text{atmospheric pressure} + 5$, and $p_4 = \text{atmospheric pressure} - h_4 = \text{atmospheric pressure} - 5$). However the difference between the pressures at any two points is equal to the hydrostatic pressure of the whole column of liquid between the two points. For instance, $p_1 - p_4 = \text{atmospheric pressure} + 5 - (\text{atmospheric pressure} - 5) = 10$.

In figure 1 (a, b, and c) there is no pressure in the system except hydrostatic pressure but in the veins there is a positive pressure, even in the dead animal, due to the contracted state of the blood vessels. In constructing a model analogous to the venous system in an animal one must consider the elastic tube system filled with fluid at a positive pressure. Take the same tube, 30 cm. long, lying horizontal and filled with liquid under a positive pressure (*i.e.*, greater than atmospheric) equal to 5 cm. of water, so that the membranes at the ends bulge. If manometers are introduced at various points in this tube the fluid in all of them will stand at a point 5 cm. above the middle of the tube (fig. 1d). If this tube is then put in a vertical position, the fluid in all the manometers will stand at a point 5 cm. above R.P., the reference point for hydrostatic pressure measurement in the tube, and $p_3 = \text{atmospheric pressure} + 5$ (existing positive pressure) $+ h_3$ (hydrostatic pressure) (fig. 1e). In this system the level of atmospheric pressure in the elastic tube system lies at the level given by the top of the fluid in the manometers but the reference point for the hydrostatic factor is below this level by an amount equal to the existing positive pressure.

This physical system (fig. 1d and e) has been found to be analogous to the venous system in the dog. In the dead animal the fluid in all the manometers comes to rest at the same level, indicating that the existing positive pressure is the same throughout the system. In the living animal the existing positive pressure is different at every point and the blood is in motion, but each point can be treated hydrostatically, according to the models given in figure 1 (d and e) to determine the reference point. In both living and dead animals R.P. is found by subtracting the existing positive pressure, as determined in the horizontal position, from the level of the manometers in the vertical position.

METHOD. Dogs, anesthetized with ether by connection of trachea to Woulffe bottle or by intratracheal blast, were firmly attached to a tilting table, after exposure of selected veins for cannulation. Glass cannulas were then inserted into superficial or deep veins, and were next attached by rubber tubing to a manometer-reservoir system, filled with 4 per cent sodium citrate solution and arranged with three-way stopcock so that the citrate solution could be run into a 1 mm. open-end manometer and thence into vein. This overflow method, with introduction of minimal amounts of fluid, allowed determination of the venous pressure at the level at which the fluid came to rest.

The connections of cannulas with the manometer-reservoir system were arranged so that the animal could be tilted from the horizontal to the two vertical positions (head-down, head-up). Furthermore the manometers were of such length (about 1.6 m.) as to permit readings of venous pressure in all positions of the animal.

In a few experiments, a cannula of 16 cm. length was inserted various distances toward the right auricle through the external jugular vein. In still other animals temporary ligations of the abdominal aorta just cardiac to the bifurcation were made, while in another group with artificial respiration the chest was opened for temporary ligation of one or both venae cavae.

The customary experimental procedure consisted in the initial determination of the pressures of the selected veins in the horizontal position of the animal and subsequent tilting of the animal to the two vertical positions with recording of the altered venous pressures. After these steps were taken, special procedures were undertaken, and on death of the animal the venous pressure readings were repeated both in the horizontal and vertical positions.

Results (dead animal). Before taking up the live animal it is well to consider the results of observations in the dead animal, which was found to be a simpler system than the living and similar to the models given in figure 1 (d and e). In the dead animal there is an existing positive pressure in the horizontal position which, when corrected for the hydrostatic positions of the various cannulas, was found to be the same throughout. The venous system was observed to act as an unbroken column from head to tail and, as the elasticity of the system is approximately the same at the two ends, one would expect to find the hydrostatic reference point near the middle of the animal.

The results can be given most clearly by considering the findings on one animal in detail. In this animal (dog D) cannulas were introduced into a superficial vein of the hind foot, into a branch of the femoral and into the external jugular. The jugular cannula was a fine brass tube which could be pushed down toward the heart so that readings were taken at different levels (C_1 , C_2 , C_3). In the horizontal position the fluid in all the manometers stood at the same level, 105 mm. above R.P., the hydrostatic reference point, which was taken as the middle of the animal in the horizontal position.² Therefore the existing positive pressure in the venous system was equal to 105 mm. of citrate. The foot vein cannula stood 20 mm. below R.P. so that the measured pressure in the foot vein (125 mm.) was made up of 105 mm. existing positive pressure and 20 mm. hydrostatic pressure. Similarly in the femoral the total pressure = 120 mm., the existing pressure

² The results were worked out assuming different points for R.P. in the horizontal animal and the midpoint was found to give a correct value for the existing pressure when the results were subsequently applied to readings in the vertical positions.

= 105 mm. and the hydrostatic pressure = 15 mm. In the jugular, where the total pressure was found to be 92 mm. and the existing pressure = 105 mm., the hydrostatic pressure was - 13 mm. of citrate.

When the animal was tilted into the two vertical positions (head-up and head-down) the fluid in the manometers stood as shown in figure 2. In the head-up position (fig. 2a) fluid in the jugular manometer in position C_3 , the femoral manometer B , and the foot vein manometer A , came to equilibrium at the same level (555 on scale) and therefore these manometers were recording correctly the pressure in the same fluid system. As the existing positive pressure is 105 mm., R.P., the level from which the hydro-

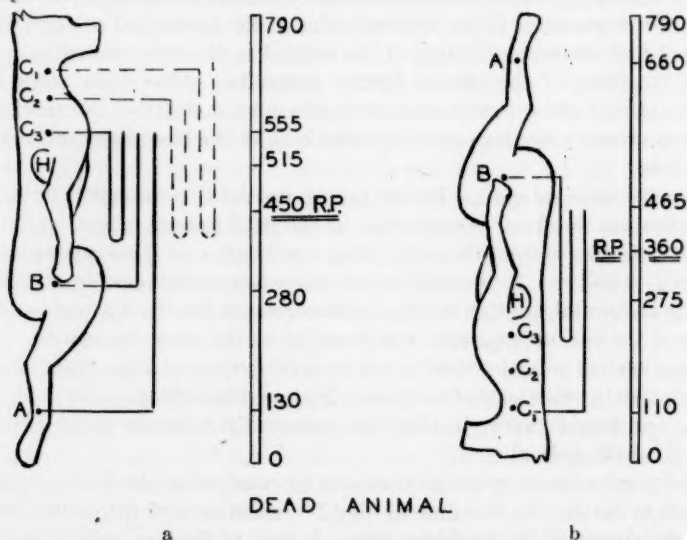


Fig. 2. Results obtained in dog D (dead) in vertical head-up and head-down positions.

static factor is measured is $555 - 105 = 450$ on the scale. This point is 450 mm. from the toes, 340 mm. from the head and 65 mm. caudal to the right auricle.

In this head-up position the readings noted for the jugular manometer in positions C_1 and C_2 were obviously incorrect and must be neglected. If correct readings were obtained the level would be 555 as in the other manometers and the difference in pressure $B - C_1$ and $B - C_2$ would be equal to the hydrostatic columns between these points. The recorded pressure in the femoral manometer, B , is $555 - 280 = 275$ mm. of citrate. At C_1 the recorded pressure is 10 mm. and the difference in pressure ($B - C_1$) is there-

fore 265 mm. whereas the hydrostatic column between these two points is $690 - 280 = 410$ mm. Similar calculations can be made for C_2 . Apparently a cannula inserted into an exposed vein will not register negative pressures correctly, owing, presumably, to collapse of the wall when exposed directly to the atmosphere. This is an important point in regard to subsequent interpretation of measurements made in the living animal.

In figure 2b (head-down), on the same basis of reasoning, the foot manometer must be neglected owing to collapse of the foot vein in this position but the femoral and jugular, C_1 , both register correctly as fluid stands at the same level in both. The hydrostatic reference point is found by subtracting the existing positive pressure from this level or $R.P. = 465 - 105 = 360$ mm. on the scale. This point is 360 mm. from the head, 430 mm. from the toes, and 85 mm. caudal to the heart. The fact that two manometers on different sides of the heart register at the same level shows that one is dealing with an unbroken column from head to toe. The reference point should be the same on the two tilts so the average gives $R.P. = 75$ mm. from the heart.

Similar measurements made on 5 dead animals, all the same size and sewed on the tilt-table in approximately the same position, give very consistent results for the position of R.P. The animals had an average overall length on the tilt-table of 820 mm., an average spinal length of 500 mm., and an average length from head to heart equal to 300 mm. Readings made with hind foot, femoral and jugular cannulas gave an average value for the position of R.P. 82 mm. caudal to the heart, the results on individual animals being respectively 85, 80, 80, 75 and 90 mm. This point was 0.54 of the distance from hind foot to nose for animals stretched out upon a tilting board in the position used in these experiments.

Results (living animals). In the living animal there are many possible complications. When the blood is flowing there is a velocity pressure in addition to the existing positive pressure and the hydrostatic factor. This velocity pressure was proved to be a negligible factor by the following experiment. After taking readings in the superficial vein of the hind foot in the horizontal, head-up and head-down positions, the abdominal aorta was ligated just central to the bifurcation into the two common iliacs. There was no blood flow in the hind leg and the horizontal venous pressure fell from 115 mm. to 82 mm. However, the venous pressure-change on tilting from the horizontal to the head-up position was the same, being respectively $+ 267$ mm. in the intact animal and $+ 270$ mm. after ligation of the abdominal aorta.

In the dead animal the initial positive pressure would not be expected to change on tilting and, although it falls slowly for some time after death, it soon reaches a relatively steady point. Therefore the value to be subtracted from the manometer readings to give R.P. would not vary in going

from the horizontal to the vertical position. In the live animal the initial positive pressure, which is due to the contraction of the blood vessels and to the residuum of pressure from the heart beat, might vary greatly with posture as the result of vasomotor reflexes affecting the arterioles and capillary bed. If there were any great difference in the initial positive pressure on tilting it would be impossible to tell what value to subtract from the manometer readings and R.P. would be indeterminate. However two experiments made to test the effect of nervous control on the initial pressure showed that there was no significant difference in the change of venous pressure on tilting when the cord was tied at the third thoracic or completely destroyed. Animal 5 was laminectomized, cannulas were put in the hind leg vein and a superficial facial vein and venous pressure-changes on tilting were taken with the animal intact and after ligation of the cord at the 3rd thoracic. Animal 10 was laminectomized in the lumbar region, a wire

TABLE 1

CANNULA	CONDITION	HORIZONTAL VENOUS PRESSURE	HEAD UP		HEAD DOWN	
			Venous pressure	Pressure change	Venous pressure	Pressure change
		mm.	mm.	mm.	mm.	mm.
Facial	Intact	70			335	+265
	Cord ligated	48			315	+267
Hind foot	Intact	176	475	+299		
	Cord ligated	162	452	+290		
Hind foot	Intact	106	430	+324		
	Cord destroyed	102	423	+321		

instrument was passed up the spinal cord destroying it to the middle of the cervical region, and both vagi were cut. Readings of venous pressure-change in the foot vein, before and after the cord was destroyed, showed no significant difference. (See table 1.)

It is clear from these observations that in the live animal the venous pressure-change on tilting is due only to a change in the hydrostatic factor and R.P. therefore may be found as in the dead animal. However in the dead animal the venous system acts as an unbroken column and in the live animal the column is interrupted at the heart. This is evident from the following experiment. With a cannula in the foot vein the pressure-change on tilting was not affected materially by ligating the superior vena cava cardiac to the azygos. And, similarly, with a cannula in the jugular there was no significant difference in the venous pressure-change on tilting when the inferior vena cava was ligated (with the azygos tied off). As ligation of these great veins close to the heart does not affect the pressure-change on

tilting the column must already be interrupted at the heart. Another point which shows that the live animal differs from the dead animal (fig. 2b and fig. 3b) is the fact that the venous pressure in the jugular (head-down) was 212 mm. in the animal when alive and 355 mm. after death. In the live animal the hydrostatic pressure is obviously measured from near the heart and in the dead animal from near the midpoint. It was at first confusing to find that the venous pressure-change in the foot vein on tilting was almost the same before and after death. This similarity of pressure-change was found to be due to the fact that in the dead dog the reference

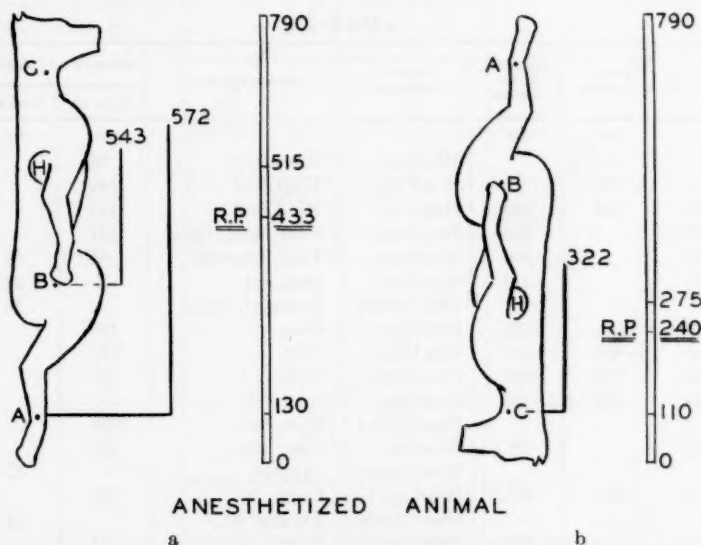


Fig. 3. Results obtained in dog D (anesthetized) in vertical head-up and head-down positions.

point is near the middle of the animal and in the live dog it is 0.8 way from hind foot to heart: these two points practically coincide.

The live animal must, therefore, be considered in two sections. On anatomical grounds one would expect the elasticity at the two ends (heart and extremities) to be different and the reference point should be nearer the heart in both sections. The hydrostatic reference points can be determined just as in the dead animal but as the existing positive pressure is different at each cannula the fluid in the manometer will not stand at the same level. The results may be analyzed in dog D again. In the live dog, in the horizontal position, manometer A (foot vein) registered a total pressure of 155 mm., which was made up of 20 mm. hydrostatic pressure (as

in dog D dead) and 135 mm. existing pressure. The femoral manometer *B* gave total pressure 128 mm., hydrostatic 15 mm. and existing pressure 113 mm. The jugular manometer *C* gave 70 mm. total, of which — 13 mm. was hydrostatic and 83 mm. existing pressure. In figure 3 the levels of the manometers are shown in the head-up and head-down positions. When the initial pressures are subtracted to give R.P. in the head-up position (fig. 3a) the femoral manometer gives $543 - 113 = 430$ on the scale and the foot manometer gives $572 - 135 = 437$. Therefore the average locates R.P. at 433 on the scale which is a point 82 mm. caudal to the heart. In the

TABLE 2

OVERALL LENGTH	SPINAL LENGTH	DISTANCE HEAD-HEART	VERTICAL POSITION	VENOUS CANNULA	DISTANCE R.P. TO HEART	
					Tail end	Head end
mm.	mm.	mm.			mm.	mm.
	475		Head up	Hind foot	98	
	485		Head up	Hind foot	149	
845	504	280	Head up	Hind foot	148	
897		280	Head up	Foot, fem., renal	121	
860		360	Head up	Foot, brachial	95	55
830		370	Head up	Brachial		34
			Head down	Brachial, facial		56
830		305	Head up	Foot	130	
803	475	285	Head up	Foot	110	
830	555	270	Head up	Foot	96	
850	550	300	Head up,			
			Head down	Foot, fem.	149	
790		290	Head up	Foot, fem.	82	
			Head down	Jugular		35
810	445	265	Head up	Foot	160	
			Head down	Jugular		31
820		295	Head up	Foot	92	
			Head down	Jugular		20
920		275	Head up	Fem.	148	
Average.....					121	38

head-down position there is only the jugular cannula which gives $322 - 82 = 240$ on the scale as the position of R.P. which is 35 mm. cephalic to the heart.

The live animal therefore has two hydrostatic reference points and averages from all the animals used (table 2) gives one 121 mm. caudal to the heart for the column from tail to heart, and one 38 mm. cephalic to the heart for the column from head to heart. With the animals stretched on the tilting board these points were about 0.8 of the distance to the heart in each column.

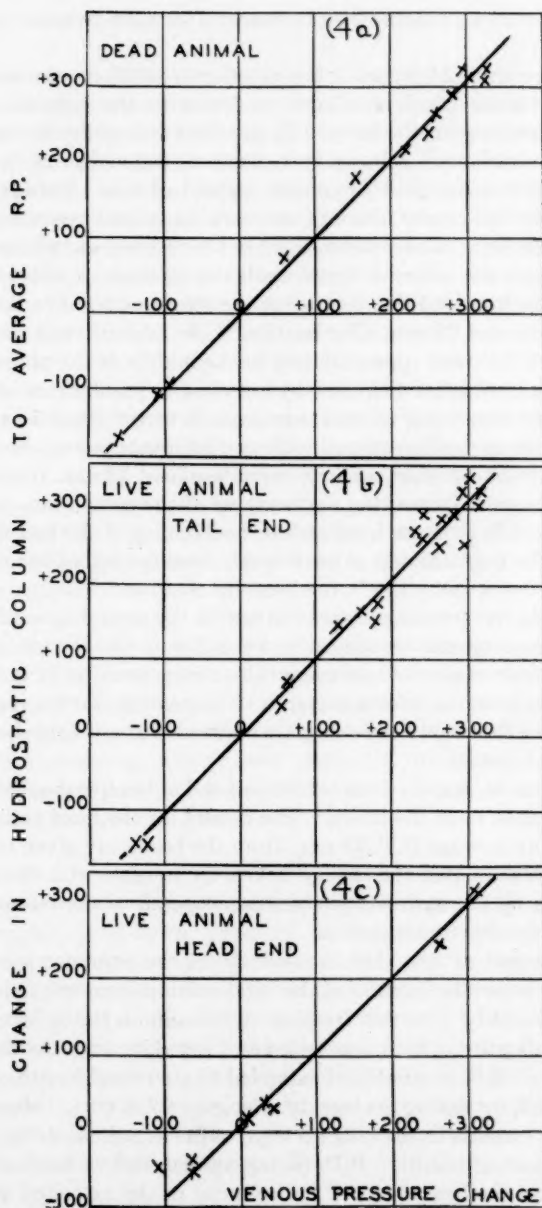


Fig. 4. Change in venous pressure on tilting from horizontal to vertical (head-up and head-down) positions plotted against change in hydrostatic column calculated to the average reference point (a) dead animal, (b) live animal, tail end. (c) live animal, head end.

In the live animal ligation of the superior or inferior vena cava does not affect the venous pressure-change on tilting as the column of blood is already interrupted at the heart. In the dead animal ligation at the heart breaks the continuous column from head to tail and gives two reference points, located as in the live animal, instead of one. This point can be illustrated by the results found in one very complete experiment. In this animal, when alive, the reference point in the tail end was 92 mm. from the heart and was not affected significantly by ligation of the inferior vena cava. In the head end the distance of the reference point to the heart was 20 mm. before and 32 mm. after ligation of the inferior vena cava. In the dead animal R.P. was approximately in the middle of the animal, 90 mm. from the heart, whether measured by hind foot or jugular manometer. On ligation of the superior or inferior vena cava, however, the dead animal gave results similar to the live animal with two reference points, one in the tail end 90 mm. from the heart, and one in the head end 32 mm. from the heart.

One of the most interesting verifications of the conclusion, in regard to the position of R.P. in the head end of the live dog, is the fact that, with a cannula in the brachial vein at heart level, there is a fall of venous pressure in the head-down and a rise in the head-up position.

In figure 4a the venous pressure-changes in the dead dog on tilting (horizontal to head-up and horizontal to head-down) are plotted against the changes in hydrostatic column using the average position of R.P. (82 mm. caudal to the heart) as reference point. The readings for the jugular head-up and for the foot vein head-down are omitted as these were shown (fig. 1) to record incorrectly.

In figure 4b the same is done for the tail end of the live dog using as R.P. a point 120 mm. from the heart. The results for the head end of the live dog, taking an average R.P. 38 mm. from the heart, are given in figure 4c. These figures show that the change in venous pressure with change in posture is given by the hydrostatic column measured to the reference points determined by this investigation.

It was thought at first that the position of the reference point was determined by a certain fraction of the total column from tail to head in the dead animal and by a certain fraction of the column to the heart in a live animal. This point of view was tested and found incorrect by determining the position of R.P. in an animal extended to an overall length of 920 mm. and shortened, by flexing the legs, to a length of 715 mm. Measurements made with a cannula in the femoral vein, with the animal living and dead, gave the following results. R.P. (living animal, tail to heart column) = 148 mm. from the heart (0.77 of the column) in the extended animal and also = 148 mm. (0.65 of the column) in the shortened animal. R.P. (dead animal) = 115 mm. caudal to the heart (0.57 of total column head to tail) in the extended animal and = 110 mm. from the heart in the shortened

animal. This seemed to prove conclusively that R.P. lies at a definite point in the animal irrespective of its overall length and should be designated by the distance from the heart, not by the fraction of the column.

There was very little variation in the position of R.P. in the dead animal but in living animals (table 2), of approximately the same size, the reference point in the tail end varied from 82 to 160 mm. from the heart. This seems to be related to a difference in the existing positive pressure as four animals with existing positive pressure in the foot vein of 90 mm. of citrate gave an average R.P. 95 mm. from the heart while four with an average existing pressure in the foot of 145 mm. of citrate gave an average R.P. 145 mm. from the heart. As the elasticity of a vein varies with the internal pressure of the fluid in it and as the position of R.P. depends on the relative elasticity of the different parts of the venous bed, it is reasonable to assume that the position of R.P. would vary with the existing positive pressure. If R.P. is at a distance from the heart approximately equal to the existing positive pressure in the superficial veins of the leg, blood from these veins would reach the heart at atmospheric pressure and a variation of R.P. with existing pressure may act as a regulatory mechanism of considerable significance.

Results with other animals. Experiments were carried out on two cats which showed that the hydrostatic reference points were similarly situated in this animal. The average overall length was 700 mm. and the distance from nose to heart was 208 mm. In the dead animal the average position for R.P. from jugular and femoral readings head-down and from foot and femoral readings head-up, was 92 mm. caudal to the heart. In the live animal the reference point, in the tail end was 127 mm. from the heart and, in the head end, 55 mm. from the heart.

SUMMARY

Direct measurements of the venous pressure in dogs in the horizontal and two vertical (head-down, head-up) positions have shown that the heart is not the point from which the hydrostatic factor in venous pressure is measured. In the dead animal the venous system acts as an unbroken column from head to tail and the reference point from which hydrostatic pressure is measured was found to be 82 mm. caudal to the heart in the vertical position in an animal of approximately 500 mm. spinal length. In the living animal the venous system is broken at the heart giving two columns with a reference point approximately 121 mm. from the heart in the tail section and another reference point 38 mm. from the heart in the head section.

THE CORTICO-FUGAL PATHWAYS MEDIATING THE "BERÜHRUNGSREFLEXE" OF MUNK AND THE CONTACT PLACING REACTIONS OF RADEMAKER¹

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The "Berührungsreflexe" of Munk, the flexion and elevation of a limb produced by gentle stroking of the hairs on the dorsum of the feet, have been shown to be cortical reflexes. They are totally and permanently abolished by removal of the motor area (Munk, 1896, Dusser de Barenne, 1933). The contact placing reactions of Rademaker (1931) (included in the group of Stehberetschaft), normally elicited by contact of the dorsum of the feet against a table edge, are also permanently abolished by removal of the motor area in cats (Bard, 1933) and in rats (Brooks, 1933). The problem here presented is, are these reflexes mediated through the pyramidal tract exclusively, or do other efferent tracts which descend from the motor area, e.g., the cortico-nigral, cortico-rubral, participate in them as well.

In a series of cats operated on with the usual surgical technique and general anesthesia, 12 bilateral and 5 unilateral lesions were made in the pyramids in the medulla through an incision in the ventral aspect of the neck, after the method of Starlinger (1895, 1897). The lesion was, therefore, well below the termination of the tracts mentioned above but involved the corticospinal fibers and injured to a varying degree the medial lemniscus. The bilateral operated animals represent the most crucial cases, since not all of the fibers cross in the decussation. Of these, five were shown, after anatomical investigation, to have a complete interruption of both corticospinal systems. The remaining had lesions of varying size down to approximately 50 per cent of the descending fibers.

In all these animals the Munk's "Berührungsreflexe" and the contact placing reactions of Rademaker were completely abolished initially. In the unilaterally operated cases, the homolateral limbs recovered completely as a rule within the first few days. In the contralateral limbs in all cases, a much slower recovery took place. A small amount of return of these reflexes was observed in from 4 to 21 days, but at the end of the period of

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observation (which varied from 2 to 3½ weeks) a certain defect was still present in that the reactions were less extensive and brisk than those on the normal side. In two cases with very extensive lesions no recovery at all was evident within three weeks and in the two with the smallest lesions, recovery was unusually rapid and appeared to be complete. The anatomical findings were carefully checked by the Marchi technique.

Since the Marchi method requires a relatively short period of survival it was not possible to determine in the cases above whether a greater or perhaps even a complete recovery might not have been possible eventually. In two unilaterally operated animals, still surviving after a period of eight months, there remains a slight but definite defect in the contralateral limbs.

The conclusions to be drawn from these animals are that lesions of the cortico-spinal tract produce a serious disorder in the "Berührungsreflexe" and the contact placing reactions; that these deficiencies are not as complete as those following removal of the motor cortex; and that some other corticofugal pathways must be capable of mediating these reactions to some extent. The second problem now arises, which are these tracts? A complete answer cannot as yet be given, but evidence is present from one animal with a complete anatomical study to show that the rubro-spinal tract is involved.

In this animal a lesion was made in the lateral aspect of the midbrain, approached through an opening in the calvarium over the occipital region. The lesion completely interrupted the left rubro-spinal tract caudal to its decussation and did not invade the cortico-spinal system. The medial lemniscus arising from the contralateral (right) side of the body was also involved. The lesion produced a temporary abolition of the "Berührungsreflexe" in all four legs. On the contralateral side the reactions rapidly returned to normal, while the homolateral limbs made only a slow recovery. Feeble "Berührungsreflexe" appeared in these after 4 days; at the end of one week the responses were still definitely reduced as compared with those on the contralateral side; at the end of two weeks no definite retardation was apparent.

An unexpected finding in this case was the fact that the contact placing reactions of Rademaker during the phase of recovery, did not parallel the "Berührungsreflexe." These responses were initially abolished, but by the 3rd and 4th days the movements on the homolateral side were excessive and jerky, i.e., hypermetric, as compared with the apparently normal, smooth and graceful reactions in the contralateral limbs. At the end of one week the same differences on the two sides were present. In the later stages, on account of the great activity of the animal, reliable comparisons could not be made. Professor Dusser de Barenne has communicated to me that he has recently observed another example of dissociation of these two reflexes, namely, the existence of apparently normal "Berührungsre-

flexe" with absence of the placing reactions in the same limbs in a decerebellate cat.

The pathways utilized by the "Berührungsreflexe" from the motor cortex to the red nucleus are not indicated in the present experiments, but at least two are available: 1, the cortico-thalamo-rubral system of Probst, which was thought by Rothmann (1901) to be one of the pathways capable of mediating the responses on electrical stimulation of the motor cortex, and 2, the direct cortico-rubral system of Monakow (1909-10).

The significant results of this investigation can be stated as follows. Lesions of the pyramidal tract give rise only to temporary abolition with some permanent deficiencies of the "Berührungsreflexe" and the contact placing reactions. Ablation of the motor area of the cerebral cortex has been shown to abolish permanently both of these groups of reflexes. These two facts taken together prove that extirpation of the motor cortex produces greater functional damage than section of the pyramidal tract originating in that cortex, and indicate that other cortico-fugal pathways must cooperate in mediating these reflexes.

The observations given above for the effects of section of the rubrospinal tract point to the cortico-rubral connections as forming one of these co-operating mechanisms.

SUMMARY

1. The "Berührungsreflexe" of Munk, and the contact placing reactions of Rademaker, permanently abolished after removal of the motor area in the cat, are less completely affected by section of the pyramidal tracts alone. They reappear, although impaired, after a few days. Therefore, remaining cortico-fugal pathways must be capable of mediating these reflexes to some extent.

2. Evidence is presented suggesting that cortico-rubrospinal system forms such a path for the "Berührungsreflexe."

3. A case of dissociation between the "Berührungsreflexe" and the contact placing reactions of Rademaker is presented.

4. In the normal carrying out of these specific reflexes which pass through the motor cortex, the pyramidal tract and other cortico-fugal mechanisms cooperate.

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INHIBITION AS AN ACCOMPANIMENT OF THE KNEE JERK

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Dusser de Barenne (5) and Buytendijk (2) first observed that a series of small irregular action currents could be detected in an extensor muscle of a decerebrate animal. Fulton and Pi-Suñer (8) and Denny-Brown (3) repeated this observation and found that if slight stretch were applied to the muscle a continuous series of action currents could be obtained from it. They found further that if tendon jerks were elicited from the stretched muscle, the typical electric response preceded the mechanical response of the jerk, but that during the mechanical response the action currents, which were present before the tendon was tapped, were absent.

This period of quiescence of the electrical record during the mechanical response of the jerk has been referred to as the "silent period" of the jerk. It should be made clear at the outset that this is merely a descriptive term, and whenever referred to in this paper will mean the entire period of quiescence which exists from the last oscillation of the electric response of the jerk to the first wave to reappear in the electrical record.

Fulton and Pi-Suñer (8) believed that the absence of action currents during the mechanical response of the jerk was due to a slackening of passive tension on receptor organs in the muscle, with the consequent cessation of activity due to the stretch reflex.

Denny-Brown (3) (4) in a series of experiments has shown that the "silent period" was not due entirely to the absence of passive stretch upon certain receptors, since he demonstrated that the "silent period" will occur though shortening of the muscle is not permitted. Likewise his records indicate that the "silent period" may end before the myograph record shows relaxation to have taken place. He concluded that the "silent period" was due to inhibition imposed upon spinal centers by proprioceptive end organs in the muscle spindles which are stimulated by the excitation wave responsible for the jerk.

Recent investigations by Matthews (10) (11) (12) have made it appear unlikely that either the hypothesis suggested by Fulton and Pi-Suñer or that advanced by Denny-Brown is entirely adequate to explain the "silent period."

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The present investigation was undertaken with the hope that by applying a technique devised by Adrian and Bronk (1) for studying the response of single motor units, further light might be thrown upon the nature of the "silent period." It was felt that if as Denny-Brown (3) concluded and as Fulton and Pi-Suñer (8) admitted might be the case, autogenous inhibition was responsible for at least part of the "silent period," the inhibitory portion might be determined in normal human subjects and be made to serve as a basis of comparison for further work with clinical cases of neuro-muscular disorders.

METHOD. For elicitation of the knee jerk and a study of the phenomena associated with it in man, the subject was seated comfortably in a chair with one leg slung over a padded cross-bar, so that the foot, when swinging, just cleared the floor. A rigid obstruction was placed in front of the ankle, so that with slight voluntary effort (extension) the ankle was brought up against it. By voluntary extension it was thus possible to produce any desired amount of electrical activity in the quadriceps group, to serve as a background against which the "silent period" might be seen to express itself. The isometric nature of contractions produced by extension against the obstruction prevented reverberations of the jerk or other movements of the limb which might disturb the electrodes.

A signal circuit was used in most cases to indicate the moment at which the stimulation for the knee jerk occurred. The signal circuit was closed by striking with a metal hammer a small copper plate which was placed over the patellar tendon. The copper plate was insulated from the subject by means of a piece of sheet rubber. In some cases the instant of the tendon tap was indicated by an artefact produced in the electrical record.

For recording the response of single motor units in human subjects, co-axial needle electrodes of the type first introduced by Adrian and Bronk (1) were inserted perpendicularly through the skin to a depth of from one to four centimeters, depending upon the thickness of the superficial layer of fat and fascia. The needle was usually manipulated until one or more units was heard clearly in the loud speaker when the leg was slightly extended against the obstruction. An electrode holder, capable of three-dimensional adjustment, was attached to the leg so that any movement of the limb which might occur would not displace the electrodes. When the leg was slightly extended to a constant degree, causing rhythmical electric responses of one or more units, the tendon was tapped, in order to elicit a knee jerk.

When gross electrodes were used they consisted of brass plates (1 cm. x 4 cm. and 4 cm. x 4 cm.) covered with canton flannel soaked in saturated NaCl solution. The small plate was placed over the approximate motor point of the rectus femoris muscle to serve as a grid lead. The large plate was placed over the skin just proximal to the patella to serve as a ground

lead. With these electrodes, slight voluntary extension caused a background response of irregular action currents, upon which the knee jerk response was superimposed.

When cats were used they were decerebrated under deep ether anesthesia at least two hours before the experiment and their limbs fixed by means of a drill through the condyles of the femur and a clamp to the tarsus. The degree of rigidity in the extensor muscle, plus a slight stretch imposed upon it, furnished a background response of one or more motor units.

The action currents were amplified by means of a six stage, transformer-coupled amplifier² which drove either a loud speaker or a Du Bois oscillograph. The oscillations of the latter were photographed upon bromide paper by means of reflected light and an electrocardiograph camera.

RESULTS. When coaxial needle electrodes are inserted into the rectus femoris or one of the other muscles of the quadriceps group in a human subject, slight extension of the leg against an obstruction produces responses in one or more individual motor units. The electric response, or action potential, which accompanies the contraction of the muscle set up by tapping the tendon, is usually a large, slightly asynchronous discharge. As pointed out by Denny-Brown (3) the electric response of the jerk is probably caused by the slightly asynchronous arrival at the muscle of single impulses in a number of motor fibers. As a result there is a summing of the electrical effects which may be detected in the muscle upon the arrival of the motor discharge, thus causing one or more large waves, depending upon the degree of synchronization.

In figure 1 may be noted three records with different amounts of background response. In A three individual unit responses are evident as a background. These are distinguishable by their characteristic wave form and by the regularity of their rhythm. When the electric response of the jerk occurs, it will be noted that it is followed by a period of quiescence during which none of the units discharge. In record B two units compose the background, which is suppressed or delayed immediately following the electric response of the jerk. Record C also shows a period of silence following the jerk. It will be noted in this latter record that there are a number of units composing the background response and that the "silent period" is shorter than in the other two records.

The period of silence in the above mentioned records, if examined closely, will show that the rhythm of the individual units, particularly in A and B, is interrupted, so that instead of discharging during the "silent period" as they would normally do, had not the jerk occurred, their next response is delayed. If it be assumed that each unit, represented in the background

² A portable amplifier designed and constructed by E. L. Garceau and demonstrated at the Cincinnati meeting of the Physiological Society, April, 1933 by Dr. H. Davis, Dr. L. J. Saul and E. L. Garceau.

response, was discharged in the electric response of the jerk, then we might expect that after a period equal to the average response interval before the jerk, we should find that particular unit discharging again. This, however, is not the case, for after such an interval from the moment of the electric response of the jerk we find that there is a further delay of from 20 to 40 σ

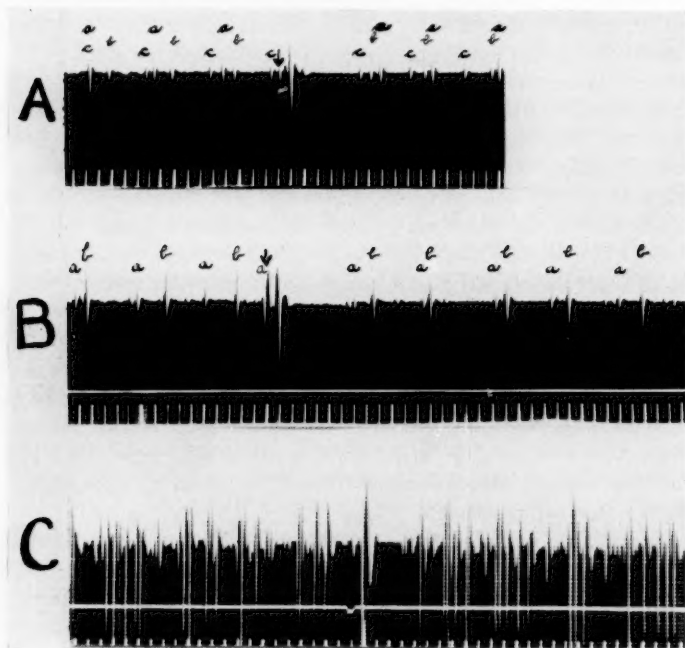


Fig. 1. Knee jerk superimposed on background of individual motor unit responses from rectus femoris muscle of a human subject during voluntary extension, showing "silent period." A. Background of three individual units *a*, *b*, and *c*; the rhythmic cycle of each is delayed by the jerk. B. Two units whose responses subsequent to jerk are delayed. C. Background of many units whose activity is suppressed for approximately 40 σ following electric response of jerk. Arrow (pointing to signal artefact) in records A and B indicates tendon tap. Same indicated in record C by deviation in signal line. Time at bottom of each record in 1/50 second intervals.

before that particular unit responds again. As will be shown subsequently, however, not every unit is discharged in the jerk response, so that the delay occasioned in such cases must be calculated from the last response before the jerk.

Denny-Brown (4) has suggested that the tendon jerk probably involves only a portion of the fibers in the muscle group stimulated. Evidence of

this has been found in several records in which the responses of individual units have been recorded. In figure 2 are shown two successive records from a series of knee jerks in which it is evident that the single unit being recorded as a background response sometimes participates in the jerk response and sometimes does not. Figure 2 A shows a single unit discharging rhythmically and then responding to the jerk stimulus just before its regular response was due. In figure 2 B the same unit, still responding rhythmically, failed to respond to the jerk stimulus applied earlier in its cycle.

With the degree of excitation (voluntary effort plus reflex effects) relatively constant, thus causing the unit to respond regularly, we may assume

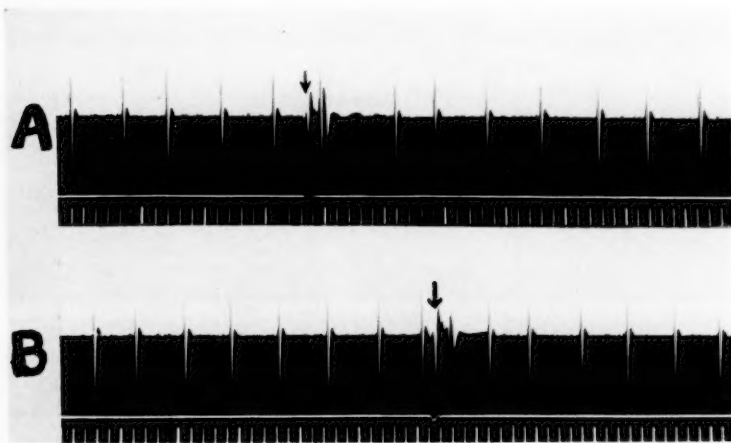


Fig. 2. Single motor unit response from rectus femoris muscle of human subject during voluntary extension, showing delay in rhythmic cycle occasioned by knee jerk. A. Unit discharged in jerk response. B. Unit not discharged in jerk response. Arrow points to artefact caused by signal circuit. Deviation in signal line indicates instant of tap on patellar tendon. Time in 1/50 second intervals.

that the observed interval between responses is that which is required for the excitatory state to be built up to the point of discharging the motor unit. Consequently, when as in figure 2 A the unit discharges in the jerk response we might expect it to discharge again at an interval after the jerk response just equal to the previous interval between successive rhythmic responses. Instead, it is consistently delayed from 20 to 40 σ , depending upon the intensity of the jerk response and also upon the degree of voluntary or reflex excitation existent at the center. On the other hand, in figure 2 B in which the unit does not take part in the jerk response, we might ex-

pect that its rhythm would continue unaltered. This is not the case for its next response after the jerk is delayed from 20 to 40σ also. Hence it appears that this 20 to 40σ interval represents a true inhibitory period apart from the interval normally required by the unit between successive responses. In a few cases the inhibitory period has been observed to be as high as 50 or 60σ , although in most cases it has been within the range mentioned above.

If this form of analysis is applied to the records shown in figure 1, we find that it holds there also and shows a similar inhibitory period for each individual unit. In figure 1 C we have noted that the number of motor units in the background is so large that individual units cannot be followed, hence it is impossible to determine the interval of each unit's rhythmic response in order to measure the true inhibitory period. Even if this were possible we may assume that when the number of units being recorded as a

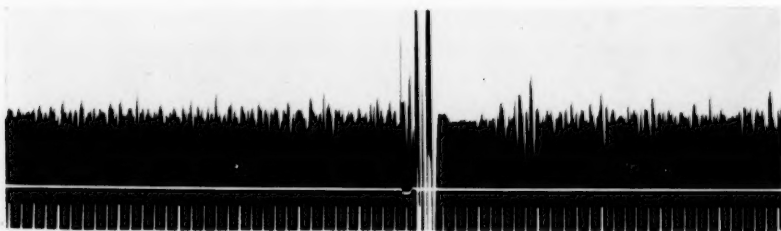


Fig. 3. Record obtained with gross electrodes over rectus femoris of human subject during voluntary extension, showing the "silent period" which occurs when the knee jerk is elicited. Deviation in signal line indicates tendon tap. Time in $1/50$ second intervals.

background response is great, the true inhibitory period could not be determined in this way, since not all of the units represented in the background response could be assumed to discharge in the jerk. As a result the rhythmic interval of some of them would have to be measured from the point where they last discharged before the jerk to their subsequent response in order to determine the delay in their rhythmic cycle occasioned by the jerk. This being the case we may then assume that whenever the background response represents a large number of units (only a fraction of which are discharged in the jerk) each discharging in random order, the "silent period" (period of quiescence from last oscillation of jerk response to the first wave to reappear in the electrical record) approximates the true inhibitory period. In other words the "silent period" which follows the electric response of the jerk in such cases is automatically limited to the period of inhibition which applies roughly to all units whether discharged in the jerk response or not. This is borne out in record C of figure 1 where the "silent period" is approxi-

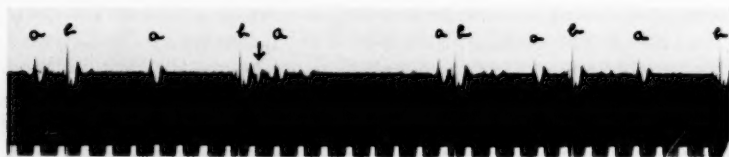
mately 40σ in duration, and is more clearly revealed in figure 3, which is a record made with gross electrodes over a muscle group and shows a background response contributed by many units (a large proportion of which presumably do not take part in the electric response of the jerk). In this latter record the "silent period" is about 40σ in duration, which compares very well with the true inhibitory period as determined in figure 2 A and B from a single unit response.

Thus to determine the true inhibitory period caused by a tendon jerk we measure the length of time the response of a single motor unit is delayed beyond its usual interval. If the unit is not discharged with the jerk the measurement is made from the last response before the jerk to its next response subsequent to the jerk. If the unit is discharged with the jerk the measurement is made from that moment to its next response. On the other hand when the background is composed of the responses of a large number of units, many of which are not discharged in the jerk response, we may assume that those not discharged with the jerk would tend to discharge in random order throughout the "silent period" were it not for the fact that each one is delayed in its response by an approximately constant amount. The tendency of a large number of units to random response plus the fact that each unit is inhibited to a similar extent, automatically limits the "silent period" in such cases to that of true inhibition.

It is interesting to note in the records presented by Fulton and Pi-Suñer (8), in which presumably a large number of units contributed to the background response, that the "silent period" as nearly as it can be determined from their records, appears to be from 20 to 50σ in duration. In some of Denny-Brown's (3) records a "silent period" of similar length may be noted, in others, however, it appears to be considerably longer. It must be remembered of course that some of those in which the "silent period" is of longer duration do not involve a background response of many units, consequently the "silent period" in such cases includes more than the true inhibitory period. If the method of determining the inhibitory interval or delay imposed upon a unit by a tendon jerk is applied to Denny-Brown's (4) (plate 12, fig. 9) record (assuming that the unit was discharged in the jerk response), the period of inhibition or delay in the unit's rhythm is approximately 40 to 50σ , which, although it is a record of a soleus jerk, is in accord with the inhibitory interval determined from our records.

Following the "silent period" the renewed rhythm of a unit is accelerated for the first few responses. The interval between the first two responses which appear after the "silent period" is usually the shortest, the unit gradually returning to its regular rate of discharge. Denny-Brown (4) has also observed this tendency on the part of a single unit to acceleration in its rate after a tendon tap (plate 12, fig. 9) or after a period of inhibition imposed upon a unit by ipsilateral stimulation of the hamstring nerve (plate 12, fig. 11).

Further support of the fact that a period of inhibition succeeds a tendon jerk response is offered in figure 4, which shows a background response of two units recorded from the sartorius muscle when slight adduction was maintained during the elicitation of the knee jerk. Although no jerk response occurs in the sartorius, the rhythmic interval of the two units following the stimulation of the patellar tendon is delayed approximately 35σ each. A subsequent acceleration of their response rhythm is also



[Fig. 4. Single unit responses from sartorius muscle of human subject showing the delay in the rhythmic response of both units following the elicitation of the jerk response in the quadriceps muscle group. Arrow points to an artefact indicating instant patellar tendon was stimulated. Time in 1/50 second intervals.

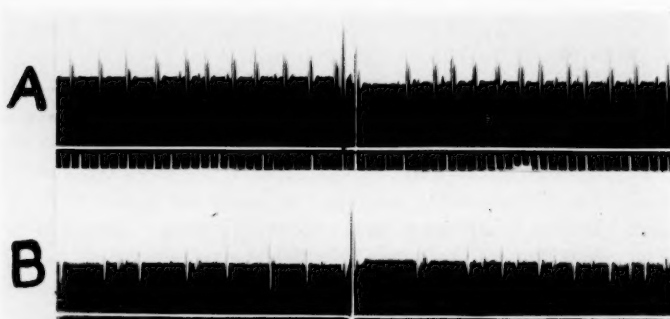


Fig. 5. Single motor unit responses produced by slight stretch upon quadriceps group of a decerebrate cat. A knee jerk response is superimposed upon the stretch reflex background causing a "silent period." A. Electrodes in rectus femoris muscle. B. Electrodes in vastus lateralis muscle. Deviation in signal line indicates tap on patellar tendon. Time in 1/50 second intervals.

noticeable. Denney-Brown (3) has likewise reported the tendency for a "silent period" to be transmitted from one extensor muscle to another, although both may not be represented in the tendon jerk response.

Similar phenomena have been found in a series of records secured from the quadriceps group of a decerebrate cat by means of coaxial electrodes. Figure 5 shows two records involving background responses of one or more units. Following the elicitation of the knee jerk in figure 5 A, which is

recorded from the rectus femoris muscle, there is a "silent period" from which may be determined an inhibitory interval of from 20 to 30 σ (assuming that the unit recorded in the background response discharged with the jerk). In figure 5 B, recorded from vastus lateralis, an inhibitory interval of similar duration may be calculated. Thus we see that whether the background response is maintained by voluntary effort in the human or by stretch reflex in the decerebrate cat, it may be abolished for a short interval during which an inhibitory state set up by the jerk is dominant at the center.

DISCUSSION. As pointed out in the introduction, there are divergent opinions as to the nature and cause of the "silent period." Fulton and Pi-Suñer (8) although admitting the possibility that autogenous inhibition might play a part, ascribed the effect mainly to the removal of the stimulus for the stretch reflex during the shortening of the muscle in the jerk response. They advanced reasons for believing that the muscle spindles are responsible for the excitatory effects which cause myotatic responses, such as are observed in the knee jerk and other forms of the stretch reflex, whereas the Golgi end-organs in the tendon were thought by them to have an inhibitory function. Denny-Brown (3) (4) on the other hand believed that the "silent period" was due to inhibition. He attributed the inhibitory effect to the muscle spindles and the excitatory function to the tendon organs of Golgi.

As shown by Denny-Brown (3) and in this paper, the absence of action currents immediately following the electric response of the tendon jerk is not due to a lack of adequate afferent stimulation, but rather to a central inhibitory effect. It is interesting to note that Hoffmann (9) a number of years ago arrived at a similar conclusion. He found that by tapping the Achilles tendon of a human subject and producing a jerk he could cause a "silent period" immediately following the electric response of the jerk. The background response recorded from the gastrocnemius was maintained by voluntary effort. The duration of the "silent period" measured on his record is approximately 40 to 50 σ , which is in accord with our findings.

Matthews (12) has recently made a very thorough study of nerve endings in mammalian muscle and on the basis of this work attributes the "silent period" to the absence of excitatory impulses from endings (A 1 type) in muscle spindles and to inhibitory impulses from tendon organs (B endings). He has also called attention to another receptor (A 2 type) in the muscle spindles which may have an inhibitory effect. Therefore it appears that the inhibitory effect which we have noted may have its origin in the tendon organs, the muscle spindles or both.

Of greater importance perhaps than the genesis of the sensory discharge is the nature of the central effect which expresses itself in the form of excitation or inhibition. The fact that a single motor unit (fig. 2) which may or may not be discharged in the jerk response, is delayed in its rhyth-

mic response immediately following the jerk by a practically constant amount indicates that the jerk in some manner sets up an afferent inhibitory effect, capable of suppressing the central excitatory state, which imposes its effect upon all motor neurones innervating muscles of related function, e.g., the extensors of a limb. The rhythmic building up of an excitatory state in those units responsible for the maintenance of the background response (either by stretch reflex or voluntary effort) is temporarily delayed in this process by inhibition. The relative constancy of the range of the inhibitory interval which we have determined and the manner in which it affects not only those elements of a "motor neurone pool" directly concerned but also those of centers of related function, suggests that the inhibitory effect under consideration abolishes a definite quantum of the central excitatory state.

The fact that an acceleration in the rhythm of the background response occurs when the rhythmic response of each individual unit is resumed following the "silent period" is indicative of post-inhibitory rebound (4) (7). The after-acceleration may be due to the emergence of an excitatory effect of the afferent discharge, previously overwhelmed by inhibition. Receptors with different thresholds and varying capacities for after-discharge might play a part in such a causation of after-acceleration.

In connection with the "silent period" subsequent to the knee jerk should be mentioned the work of Eccles and Hoff (6), in which they dealt with single motor units reflexly activated by stimulation of a contralateral afferent nerve and investigated the effect of an antidromic impulse in a motor neurone. They found that when an antidromic impulse acts on a rhythmically discharging motor neurone it interrupts the rhythm and alters its subsequent phase. The earlier the single antidromic impulse acts in the course of a rhythmic cycle the more it lengthens the subsequent cycle. Since these investigators have likened the effects of an antidromic impulse on a motor neurone to that of a reflex discharge, it might be assumed that the lengthening of the rhythmic cycle of a motor unit following a knee jerk in our records was due to a similar cause. If our records are examined carefully, however, it will be noted (fig. 1 A) that the delay in the rhythmic cycle of each unit subsequent to the jerk is relatively constant and is entirely independent of the curtailment of the cycle in which the electric response of the jerk falls. In contradistinction also to the findings of Eccles and Hoff that the rhythmic discharge following an antidromic impulse recommences at the same rate as before, we have found that there is usually an after-acceleration in the rhythm following a jerk response. These and other differences lead us to believe that we are dealing with central inhibition occasioned by the knee jerk, rather than an effect like that of an antidromic impulse.

Denny-Brown (4) has mentioned the tendency to synchronization of

response of two or more units following a short-lasting inhibition, and has called attention to its importance in the genesis of clonus. There does not seem to be a marked tendency toward synchronization by rebound in our records.

The apparent constancy of the inhibitory interval of the knee jerk in a human subject as determined by the single motor unit technique or with gross electrodes suggests that it may be a valuable measure in the study of neuro-muscular disorders. Likewise, variations in the inhibitory period, properly correlated with histological studies may throw additional light upon the function of various receptors.

SUMMARY

1. When single motor unit responses are recorded from the muscles of the quadriceps group of a human subject (voluntary extension) or the decerebrate cat (stretch reflex) we find that subsequent to elicitation of the knee jerk the rhythmic cycle is delayed for a period of from 20 to 40 σ , occasionally as much as 60 σ . This period of delay has been interpreted as an inhibitory interval.

2. Not all motor units are discharged in the jerk response, but whether they are or not, they are similarly delayed in their subsequent response.

3. Following the period of inhibition each unit resumes its rhythmic response at an accelerated rate, but gradually returns to its original rate before the jerk occurred.

4. The inhibitory interval extends to muscles of related function although they are not actually involved in the jerk response.

5. When the number of units composing the background response is large the "silent period" is an approximate measure of the inhibitory interval.

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